THE OFFICE LABORATORY

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THIRD EDITION
Revised and Enlarged

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To all our former students
this book is respectfully
dedicated.

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U. S. A.

PREFACE TO THIRD EDITION

In this edition of "Office Laboratory" the same general plan of compactness and simplicity has been retained as in previous editions. The average physician already has many reference books on laboratory technique, and we are making no effort to add another. The main object of this manual is to give a workable, practical laboratory guide which the office technician can follow.

No attempt is made to cover the entire field of Clinical Laboratory but simply the portion routinely used in the physician's office. Only those tests of proven merit are described. An additional chapter is added for technicians desiring to do a little more advanced work. The authors acknowledge their indebtedness to the research workers in this field of science, and express deep appreciation to all who have assisted in the preparation of this work.

Wm. W. Worster
J. Wilder Salisbury

San Gabriel, Calif.
April 1, 1938

PREFACE TO FIRST EDITION

This manual represents in printed form the notes which the Author has used as a basis of his lectures in clinical laboratory work. It has been published primarily to save the students the laborious task of note-taking. It is not intended to replace any of the standard works, but simply to present a manual of the tests routinely used in simplified form.

The Author acknowledges his indebtedness to the research workers in this field and to the standard works upon the subject. He also wishes to express appreciation for the faithful work of his staff in the preparation of the manual.

Wm. W. Worster.

San Gabriel, California
November 1, 1933

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FIG. 1 — COMPACT OFFICE LABORATORY KIT

CHAPTER I

INTRODUCTION

It is a well known fact that a clinical laboratory in order to do the best quality of work must employ only well trained technicians. Of equal importance is the necessity of up-to-date equipment. A modern microscope and a standard colorimeter must always be included. Unless specially prepared solutions are purchased an accurate analytical balance is imperative also.

The Microscope

No single instrument used in the clinical laboratory is so essential as a good microscope, and none requires greater care. For the benefit of those who are not familiar with it, the following description and suggestions are given.

Regardless of its age or make, a microscope must have proper light in order to do efficient work. A double **mirror**, flat on one side and concave on the other, is used to deflect the light. When used with direct daylight, the flat side is preferable and with artificial light, the concave side. In either case the light is thrown in parallel rays against the condenser.

A new type of lamp with a daylight window is being used quite extensively. With this new lamp, move the mirror to the side of the microscope, as the light shines directly from the lamp into the condenser. Also move the illuminator until the maximum lighting efficiency is found.

The light, as above stated, is thrown directly upon the **condenser** which converges and intensifies the rays. Adjust the condenser so as to give the proper focus upon the microscopic slide.

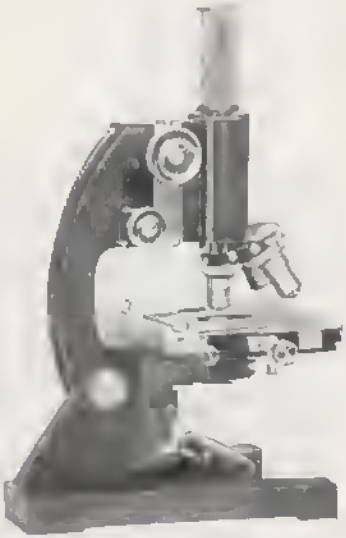

FIG. 2 MODERN MICROSCOPE

Between the condenser and the mirror or between the condenser and the slide is inserted a **diaphragm.** Sometimes one is inserted in both places on the same microscope. The diaphragm controls the quantity of light used. Satisfactory illumination of the slide then depends upon the proper source of light, the proper aperture of the diaphragm and the proper adjustment of the condenser. A little practice is necessary to master these adjustments.

Just above the condenser is a **stage**—a flat platform upon which the slide and cover slip are placed. In order to facilitate the examination, a mechanical stage is often added and some microscopes have it as standard equipment.

Above the stage and screwed to the end of the barrel is the **objective**, one of the magnifying parts. Modern microscopes have three so arranged that each may be rotated to its proper position. They are designated, according to focal distance, as 16, 4, and 1.9 mms. The one marked 16 is called "low power"; the second marked 4, "high dry"; and the third, marked 1.9, "oil immersion", since a specially prepared oil between it and the slide is required.

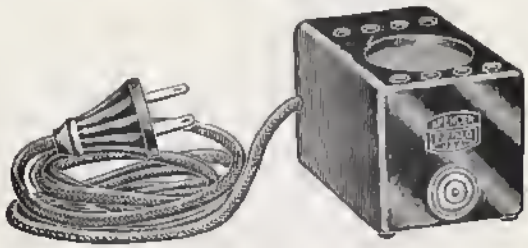

Fig. 3 Substage Microscope Lamp

At the top of the microscope is the **eyepiece**, or ocular, which aids the objective in its magnifying power. Two eyepieces usually come with each microscope. Between the eyepiece and the objective is the barrel, or body tube. For each microscope there is a certain length of barrel which is preferred, but various lengths are used.

Microscopes have two screw adjustments, coarse and fine, for focusing. The coarse one is used to find the field and the other for fine adjustments to sharpen the image.

In using the microscope the following **technique** is advised: place the slide on the mechanical stage and use the low power for preliminary study. If further magnification is needed, use either the high dry or the oil immersion as the case may demand.

In focusing, exercise great care that the objective is not screwed down too far, as the cover slip is liable to be broken and the lens damaged. Until experienced in focusing, it is better to screw the lens with the coarse adjustment until it almost touches the slide. Hold the head horizontally with the stage so as to observe when this position is reached. Now look down through the eyepiece and "focus up" until the image is seen. Then use the fine adjustment to clarify the image. Thus the danger of injuring the slide or lens is eliminated.

Cedar oil is commonly used with the oil immersion objective. Keep the bottle well stoppered as the oil thickens on exposure to air. A clear, heavy, high-grade mineral oil can be used to advantage since it does not dry on the slides nor gum the objective. Its optical properties are quite satisfactory for ordinary work.

All microscopes should be handled with great care and protected from dust when not in use. Never permit alcohol to come in contact with any lacquered part. Clean the lens with soft linen or lens paper only. Never permit oil to dry on the oil immersion lens, but if this accidentally occurs remove by using

xylol. Do not use too much xylol as it may dissolve the cement which holds the lens in position.

The Colorimeter

A colorimeter is an instrument used to compare colors of solutions. It is used for the quantitative determination of any substance which can be changed

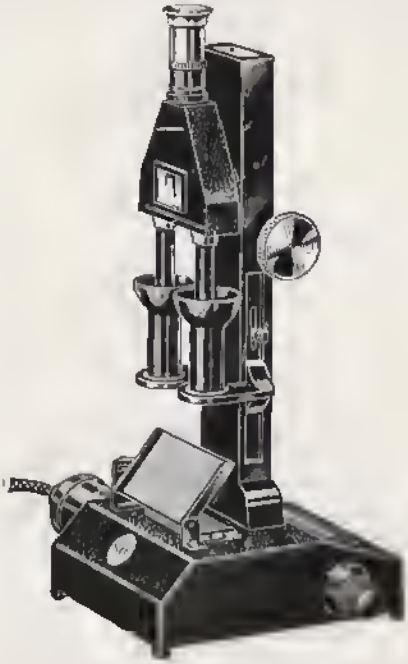

FIG. 4 — KLETT COLORIMETER

into a soluble colored compound. It is accurate and gaining in popularity. The "unknown" amount of a substance in solution is determined by comparing it with a "known" amount of compound.

There are several kinds of colorimeters but the Duboscq type is the one most generally used. It has two glass-bottomed cups that can be raised or lowered as desired. Into each cup is inserted a glass plunger. As the cup is raised or lowered the distance between the end of the plunger and the bottom of the cup varies. Each instrument has a scale to determine this distance, measured in millimeters. Beneath the cups is a mirror (or electric light) which reflects the light up through the bottom of the cups and the plungers. Then by means of a prism the light rays are bent, so, when looking through the eyepiece the light from each cup fills just half the field.

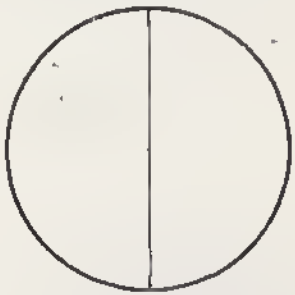

FIG. 5 — COLORIMETER FIELD

In making a determination, it would be well to have one cup (usually the one at the right) marked "U" to be used for the unknown solution, and the other marked "S" for the standard. Set the standard at some definite reading, preferably 20 or 15 mms. Adjust the "unknown" until its half of the field exactly matches the color of the standard half.

The Analytical Balance

Another instrument of precision necessary for the clinical laboratory is an analytical balance, accurate enough to weigh quantities as small as 0.1 mg. This is very essential in making volumetric solutions. Be sure it is always in perfect order. Never touch the weights with the fingers, but use forceps made specially for that purpose. Protect the instrument from moisture, dust, and chemicals, and when not in use keep case closed.

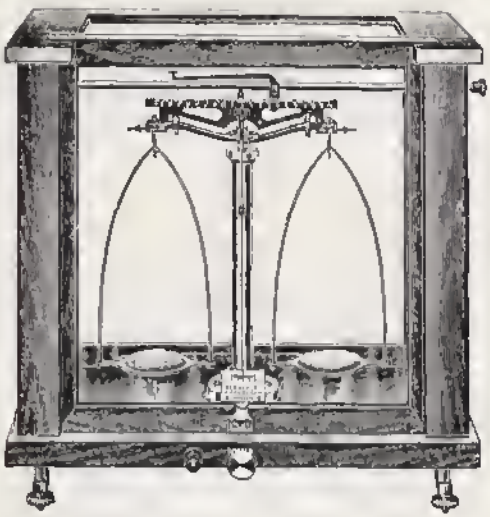

Fig. 6 — Analytical Balance

CHAPTER II

URINALYSIS

Physical and Microscopical

Urine is an aqueous solution of organic and inorganic substances which are waste or by-products of metabolism. The organic substances found are urea, uric acid, creatinin and sometimes sugar and albumin. The inorganic substances are composed mostly of salts.

Before examining a specimen, be sure to have the **full name** of the patient, or the report may be useless.

When supplying **containers** for the patient's use, have the name on the jar—not written on the sack or paper in which it is wrapped. If the patient furnishes his own container, instruct him how to mark it properly. When more than one container is brought to the laboratory by the same patient, have each one properly labeled.

In the routine examination of urine there are three divisions to be considered: physical, chemical and microscopical.

Physical

The **quantity** of urine voided varies from day to day in proportion to the amount of liquids consumed, the condition of health, and the climate. The average is 1200 to 1500 c.c.

If only a single specimen is to be examined, it may be voided at any hour during the day, but the morning specimen is preferred. For a quantitative analysis, it is necessary to have a mixed, twenty-four hour specimen. To obtain this, instruct the patient to void

and discard at the hour of rising. Save all urine from this time to and including the same hour the following morning. Bring the whole amount to the laboratory if possible, otherwise thoroughly mix, accurately measure, and remove a sample before any deposit settles.

All specimens should be brought to the laboratory as early as possible after voiding, as decomposition takes place rapidly.

In designating the **color** of the urine, it is described as pale, light-yellow, yellow, yellowish-red, reddish-brown, red, green, or black. Normally it varies between light-yellow and yellowish-red.

Urine has an **odor** peculiar to itself and unless it deviates from this, is recorded as normal. Old urine, due to decomposition, develops an ammoniacal and often a foul odor. In diabetes there is sometimes a sweetish odor due to the presence of acetone.

Urine freshly voided should contain no **deposits**. After standing, they may precipitate due to the cooling of the urine or to fermentation. The amount is expressed as slight, moderate or heavy.

Fresh urine is usually clear, but may become cloudy, with or without a precipitate. Designate the **transparency** on the chart as clear, cloudy or opaque.

The **reaction** is usually tested with litmus paper and designated as acid, neutral, or alkaline. Blue litmus paper turns red in an acid urine and red litmus paper turns blue in an alkaline urine. A mixed twenty-four hour specimen of normal urine is acid.

Individual specimens, especially voided after a full meal, may be slightly alkaline. Old urine is invariably alkaline.

The specific gravity is determined by means of a urinometer. Accurate readings are obtained only when the temperature of the urine is 22.5 or 25 degrees C. according to the make of the instrument. These are approximately room temperature. If the urine is above or below the calibrated temperature of

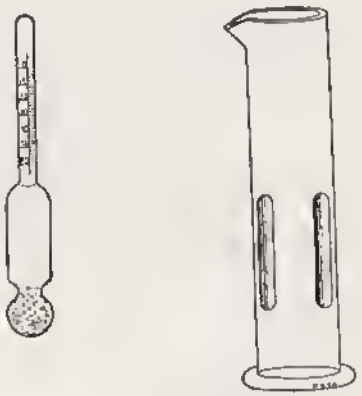

FIG. 7 — URINOMETER

the urinometer, make corrections as follows: add 0.001 to the reading for each 3 degrees C. above and subtract 0.001 for each 3 degrees C. below. Specific gravity varies directly with the amount of solids, and inversely with the volume of urine excreted by the kidneys.

Microscopical

The routine examination of the urine should, when possible, include a microscopical examination. If insufficient sediment is present, centrifuge the specimen. The sediment can best be removed by means of a pipette. A drop should be placed on a glass slide and covered with a cover slip. Many times a satisfactory examination can be made with low power without covering, but routinely it is better to cover it. Never allow the sediment to dry on the slide.

The deposit is composed of organic or inorganic substances, or both. The organic may be composed of casts, pus cells, blood cells, epithelial cells, or bacteria. An inorganic deposit consists of chemical substances, crystalline or amorphous.

Of the organic substances, **casts** are the most important. **Hyaline** casts are the simplest and probably the foundation structure for many others. They are an albuminous substance which has solidified in the tubules of the kidney and pushed out by the pressure of the urine. They represent a diseased kidney. Under the microscope they appear as semitransparent bodies about six or eight times as long as their diameter.

Sometimes varying amounts of granules are present, and form **granular** casts. Fatty substances may be deposited in the cast which would make **fatty** casts. Often, waxy casts, shorter than the hyaline casts and more opaque, are present. They are called waxy because of their appearance, but in all probability are old hyaline casts. Many times the epithelial

lining of the tubule sloughs, giving rise to **epithelial** casts.

Blood, pus and bacteria can plug the tubule of the kidney and when forced out, make casts of the corresponding name—**blood** casts, pus casts or **bacterial** casts. While casts of the latter group may occur, usually blood, pus and bacteria exist singly and not massed.

FIG. 8.—CASTS: A, HYALINE; B, WAXY; C, EPITHELIAL; D, GRANULAR; E, FATTY; F, BLOOD

Epithelial cells also may be present, floating around, having no connection with each other, or may exist closely associated, as though a portion of the epithelium had sloughed.

Of the inorganic substances, there are two main kinds—those occuring in acid urine and those in alkaline urine. Crystals resulting from fermentation have no clinical significance.

In the acid urine type, **uric acid** crystals are possibly the most conspicuous. They occur as yellow to golden colored, many-shaped crystals which either stick to the side of the jar or settle to the bottom. They often have a beautiful appearance.

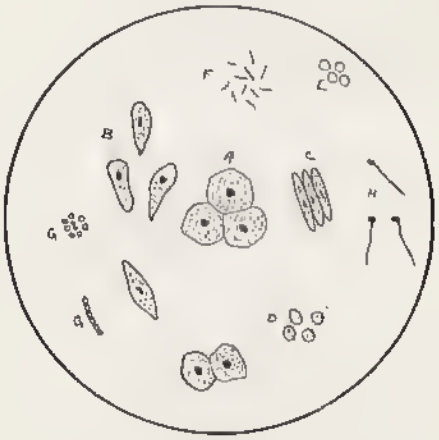

FIG. 9 A, B, C, EPITHELIAL CELLS; D, PUS; E, BLOOD; F, G, BACTERIA; H, SPERMATOZOA

Little clinical significance can be ascribed to them unless they are precipitated in large quantities soon after the urine is voided. They dissolve in both sodium and ammonium hydroxide. When the latter is used they will again be precipitated in the form of ammonium urate crystals.

Amorphous urates, a name given to sodium or potassium urate, form a very heavy granular "brick-dust" deposit. They occur in concentrated and very acid urine, especially in febrile conditions. Heating or acetic acid completely dissolves them. In a half hour, if the acid is used, they will be precipitated again as uric acid crystals.

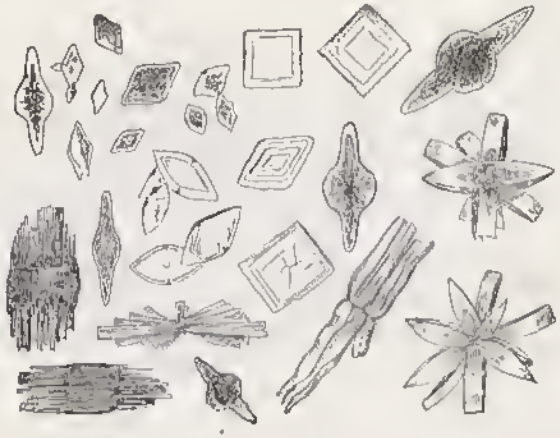

FIG. 10 — VARIOUS FORMS OF URIC ACID CRYSTALS (Ogden)

Calcium oxalate crystals are colorless, usually of a glistening nature. They have the general appearance of an envelope, although there are many modifications and varieties. When present, the urine is usually acid but occasionally alkaline. Articles of food which contain oxalic acid, such as rhubarb, spinach and tomatoes when injested increase the number of calcium oxalate crystals in the urine.

In alkaline urine are found the various deposits of phosphates, calcium carbonate and ammonium urate.

FIG. 11 VARIOUS FORMS OF CALCIUM OXALATE CRYSTALS (Peyer)

The most common phosphate is the ammoniomagnesium phosphate crystal, called **triple phosphate**. The shape of these crystals is similar to a prism with both ends sloping to the center. Sometimes they resemble calcium oxalate but are easily differentiated by the absence of a shiny appearance. If precipitated rapidly they have a peculiar shape similar to a feather. Triple phosphates are usually found in alkaline urine but may be present in acid urine if fermentation is taking place.

Amorphous phosphates are found under the same conditions as the triple phosphates and occur as granular sediment, being distinguished from amorphous urates by their lack of color and resistance to heat. They are, however, soluble in acetic acid.

FIG. 12 — 1, TRIPLE PHOSPHATE CRYSTALS; 2, AMMONIUM URATE CRYSTALS; 3, AMORPHOUS PHOSPHATES

Sodium phosphate is deposited in the urine in mild alkaline or slightly acid conditions. It has two forms, one as colorless prisms arranged like stars and the other as flat flakes resembling squamous epithelium.

Ammonium urate occurs as spherical bodies with needle-like projections on them. They are precipitated from fermenting urine by ammonia as previously mentioned under the heading of uric acid.

CHAPTER III

URINALYSIS

Chemical

The acidity of the urine is due to the presence of acid phosphates and free organic acids. It is advantageous to know the amount of acidity in diagnosing certain diseases. For the determination of total acidity, the titration and colorimic methods are the two most generally used. Always examine before fermentation starts, and be sure that no acid was used as a preservative.

The titration method uses N/10 sodium hydroxide to neutralize the acidity and the result is expressed in degrees of acidity. Zero expresses neutrality. Twenty-five degrees is approximately the normal acid reaction of the urine.

The colorimic method is based upon the hydrogen-ion (pH) concentration, and is not expressed in degrees but in figures ranging between pH 5 and pH 9. Neutrality is represented by pH 7. Any figure smaller than this indicates acidity, which increases as the figure decreases, and any number larger represents alkalinity, which increases as the figure increases. Normal urine has an average acidity of pH 6. To obtain the degree of hydrogen-ion concentration, we rely upon the properties of certain chemical reagents which change their color with different degrees of hydrogen-ion concentration.

Since no single indicator has sufficient color range to cover the required pH scale when testing urine, a **duplex indicator** solution is found convenient, consisting of methyl red and brom-thymol blue. The former is more sensitive to strongly acid urine, the latter works better with normal or alkaline urine.

La Motte Hydrogen-Ion Concentration Test

(a) Place 10 c.c. urine in a test tube of 16 mm. internal diameter.
(b) Add the required amount of the duplex indicator as stated on the bottle.
(c) Mix by gently inverting once or twice. Never shake violently.
(d) Compare this tube with the colors of the sealed standard tubes. Thus the approximate pH value can be determined.
(e) If the urine is highly colored use the compensator block which comes with the outfit.

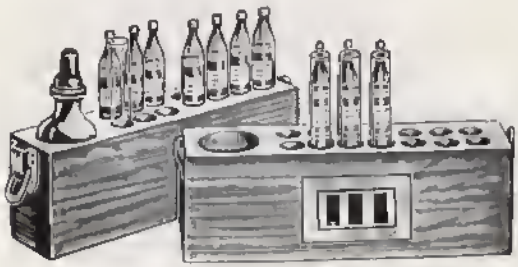

FIG. 13 OUTFIT FOR DETERMINING pH OF URINE

Test for Degrees Acidity

(a) Place 10 c.c. urine in a 50 c.c. beaker.
(b) Fill the beaker about half full with distilled water.
(c) Add 3 drops of phenolphthalein indicator.
(d) Titrate with N/10 sodium hydroxide until the first permanent faint pink color appears.
(e) The degrees acidity equal the cubic centimeters of hydroxide used, multiplied by ten.
(f) When the urine is alkaline, the indicator turns pink immediately. Titrate with N/10 hydrochloric acid until only a faint color remains.
(g) Calculate as before; the result, however, is degrees alkalinity.

The amount of **total solids** in the urine varies from 40 to 50 gms. according to age, diet, exercise and other factors. Haser has a simple and yet reliable method for determining total solids.

Haser's Method

(a) Multiply the last two figures (or the last figure if it is preceded by a zero) of the specific gravity by 2.33, which gives the grams total solids in a liter.

(b) Divide this amount by 10, which gives the grams total solids in 100 c.c. or the per cent.

(c) To calculate the grams total solids in the entire specimen, divide the number of grams found in one liter by 1000, and then multiply by the total number of cubic centimeters.

Urea is the end product of protein metabolism and constitutes the largest single factor which makes up the total solids. The amount found in the urine varies according to diet, being lower with vegetarians. The average is 30 gms. Many laboratories determine urea only when requested.

There are two methods in common use for finding the amount of urea in the urine: the urease and the sodium hypobromite. The urease method is much more accurate. The **urease enzyme** in the Jack bean meal converts the urea into ammonium carbonate. This is neutralized with hydrochloric acid. Two flasks are used, one serving as control to determine the amount of acid required to neutralize the water, urine and activated urease meal.

The sodium hypobromite method is simpler to perform but is not as accurate, since other nitrogenous compounds besides urea are decomposed, yielding an additional amount of nitrogen.

The Marshall Urease Test (Modified)

(a) Place 100 c.c. water in each of two 200 c.c. flasks.
(b) Add 100 mg. activated urease powder (or crushed tablet) to each flask.
(c) Heat one flask to boiling to "kill" the enzyme. This flask is the control.
(d) Add 5 c.c. urine and 1 c.c. toluol to each flask, and stopper.
(e) Immerse in water bath at 50°C. for one hour.
(f) Add 5 drops methyl orange solution to each flask.
(g) Titrate each solution with N/10 hydrochloric acid to the same pink appearance.
(h) Subtract the number of cubic centimeters acid required to neutralize the control flask from the other.
(i) Multiply this difference by 0.06 for the percentage urea.

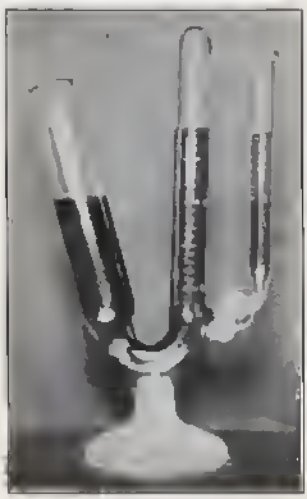

FIG. 14 — UREOMETER, DOREMUS-HINDS

Sodium Hypobromite Method

(a) Fill the middle tube of the Doremus-Hinds ureometer with freshly prepared sodium hypobromite solution.
(b) Fill the small tube to the zero mark with urine.
(c) Allow 1 c.c. urine to slowly run through the valve. The commotion is caused by nitrogen gas collecting at the top. Do not allow any of this gas to escape.
(d) The tube reads grams urea in 1 c.c. urine, which multiplied by 100 gives the per cent urea.

Sugar is not a normal constituent of the urine so, if constantly found, indicates disease. For a **qualitative** determination of sugar, there are three methods generally used: Benedict's, Haines', and Fehling's. Benedict's method is preferred as it is the most accurate.

To determine the amount of sugar, Benedict's method is also preferred, but for this test we use Benedict's **quantitative** solution.

Benedict's Qualitative Test

(a) Place 5 c.c. Benedict's qualitative solution in a test tube and boil for a few moments. If the blue color turns green or yellow the solution must be discarded.
(b) Using a freshly prepared solution, add eight or ten drops urine and boil. If no color change is noted, boil vigorously for a minute or two and cool slowly. This will detect very small quantities of sugar. A cuprous oxide precipitate is always found if sugar is present.

Benedict's Quantitative Test

(a) Place 10 c.c. Benedict's quantitative solution in a 160 c.c. beaker.
(b) Add about 25 c.c. distilled water.
(c) Add 3 gm. anhydrous sodium carbonate.

(d) Heat to boiling and keep boiling all through the test.

(e) When the boiling point is reached, add urine, slowly, with constant stirring, until the blue color turns milky. The cubic centimeters urine used contain 0.02 gm. dextrose.

(f) To determine the percentage of sugar, divide 0.02 by the cubic centimeters of urine used, and multiply by 100.

Albumin is an abnormal element of the urine, and denotes a diseased condition of the kidney. For its detection from a qualitative or a quantitative standpoint the urine must be filtered if at all cloudy. Of the several qualitative tests in use, Purdy's is probably the best.

Purdy's Qualitative Test

(a) Fill a test tube half full with urine.

(b) Add about one sixth as much saturated sodium chloride solution.

(c) Add 5 to 10 drops 50% acetic acid.

(d) Gently heat the top portion of the liquid in the tube.

(e) The appearance of a cloud indicates albumin.

Heat and Nitric Acid Test

(a) Boil 5 c.c. filtered urine in a test tube. If a cloud appears it probably is albumin or phosphates.

(b) Add not to exceed 3 drops of nitric acid. Stir gently. Nitric acid should dissolve any phosphates present, but not the albumin.

Heller's Ring Test

(a) Take a test tube one fourth full of nitric acid.

(b) Hold the tube on a slant, slowly add a little urine, allowing it to flow over the surface of the acid.

(c) Albumin forms a white ring at the point of contact.

The above tests detect only the presence of albumin. The most practical method of determining the **quantity** is Purdy's.

Purdy's Centrifuge Method

(a) Place 10 c.c. filtered urine in a graduated centrifuge tube.
(b) Add 2 c.c. 50% acetic acid and 3 c.c. 10% potassium ferrocyanide.
(c) Mix and let stand at least 10 minutes.
(d) Centrifuge at 1500 r.p.m. for 5 minutes.
(e) The tenths of cubic centimeters of precipitate is the volumetric per cent of albumin.

When found necessary to convert the volumetric per cent into the grammetric, the following table may be used:

Table showing relation of volumetric percentage (Purdy's) with grammetric percentage.

1	.021	16	.333
2	.042	17	.354
3	.063	18	.375
4	.083	19	.396
5	.104	20	.417
6	.125	21	.438
7	.146	22	.458
8	.167	23	.479
9	.187	24	.500
10	.208	25	.521
11	.229	26	.542
12	.250	27	.563
13	.271	28	.583
14	.292	29	.604
15	.313	30	.625

Another important quantitative test for albumin is Exton's. This is a turbidimetric test, which shows the milligrams protein in 100 c.c. urine by comparing with the standard tubes furnished by the manufacturer. This is a very accurate test.

The Exton Test

(a) Procure an Exton outfit.
(b) Add about 3 c.c. of Exton's reagent to an equal amount of urine in one of the tubes.
(c) Warm gently in the flame. Do not boil.
(d) Compare with the standard tubes to determine the amount of albumin.
(e) It may be necessary to use diluted urine when albumin is high, and calculate accordingly.

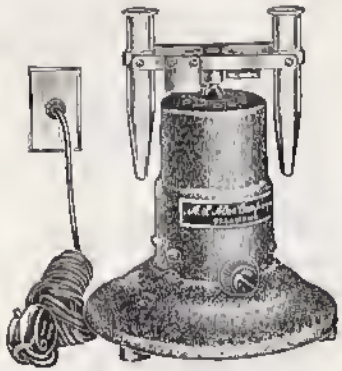

FIG. 15 — ELECTRIC CENTRIFUGE

In addition to albumin there is sometimes present in the urine Bence-Jones' protein. Its presence indicates some pathological condition.

Bence-Jones' Protein

(a) Slightly acidify the urine by adding a few drops of acetic acid.

(b) Gently heat in a water bath. Turbidity will begin approximately at 40°C. if the protein is present. When the temperature reaches 60°C., a precipitate forms. When the boiling point is nearly reached, the precipitate may entirely dissolve. Upon cooling, the precipitate reappears.

(c) The protein may be overlooked if albumin is present. In this case, remove the albumin by filtration while the solution is boiling hot.

Diacetic acid in the urine indicates a condition of acidosis which is usually present in severe cases of diabetes.

Gerhard's Test

(a) Place about 10 c.c. urine in a test tube.

(b) Add, drop by drop, 10% ferric chloride to precipitate the phosphates.

(c) Remove the precipitate by filtration and add more ferric chloride.

(d) The urine turns a port wine color when diacetic acid is present.

Certain other substances may cause the same color as diacetic acid in the test, so when in doubt, proceed as follows:

Confirmatory Test

(a) Add 5 c.c. water to 5 c.c. urine.

(b) Boil down to 5 c.c. Diacetic acid, if present, will boil away.

(c) Now add the ferric chloride, and no port wine color should appear.

Acetone occurs under the same conditions as diacetic acid and has the same clinical significance.

Rothera's Test

(a) Add 1 gm. ammonium sulphate to 10 c.c. urine.
(b) Add 3 drops of freshly prepared saturate solution of sodium nitroprusside (or about 6 drops of the stable solution).
(c) Gently overlay with strong ammonia. If acetone is present a reddish purple ring will form at the point of contact.

For the confirmatory test, boil the diluted urine as in the diacetic acid test, then proceed as before. Boiling removes acetone.

Indican is one of the by-products of putrefaction. Neither the quantity nor the toxic nature is sufficient to be a detriment to the body, yet it indicates the amount of putrefaction that takes place in the bowel.

Obermayer's Test

(a) Place 5 c.c. urine in a test tube.
(b) Add 5 c.c. Obermayer's solution.
(c) Add 1 c.c. chloroform.
(d) Mix gently. Never shake violently.
(e) Let stand for a few minutes.
(f) The chloroform turns blue when indican is present. The intensity of the blue determines the amount which is recorded as 1 plus, 2 plus, 3 plus, 4 plus.

Iodides in the urine mask the blue in the chloroform in the indican test by causing a red coloration. Remove them as follows:

To Remove Iodides

(a) Add a few drops of a saturate solution of sodium hyposulphite.
(b) Shake well. The red color caused by the iodides now disappears.

Blood in the urine indicates hemorrhage in the kidney or bladder.

Benzidine Base Test

(a) Boil the urine, and allow to cool. This destroys the oxidases.
(b) Take 2 c.c. of the urine and add 2 c.c. freshly prepared saturate solution benzidine base in glacial acetic acid.
(c) Let stand a few minutes in a test tube.
(d) Hold the tube on a slant, and slowly add a few drops of fresh hydrogen peroxide.
(e) Blood causes a greenish or bluish ring at the point of contact.
(f) Record the amount of blood as for indican.

Bile in the urine usually denotes some liver disorder. It may be found as bile pigments or bile acids.

Smith's Test for Bile Pigments

(a) Place some urine in a large test tube.
(b) Overlay with a 10% alcoholic solution of tincture of iodine.
(c) Pigments cause a green ring to appear at the zone of contact. This is seen more clearly against a white background.

Hay's Test for Bile Acids

(a) Place some urine in a small beaker.
(b) Sprinkle with a pinch of flowers of sulphur.
(c) Bile acids cause the sulphur to settle, by reducing surface tension.
(d) For a more sensitive test, gently shake the beaker. Then a trace of bile acids causes the sulphur to settle.

CHAPTER IV

GASTRIC ANALYSIS

The analysis of the gastric secretion gives many diagnostic points, although of not so much importance as previously considered.

The stomach secretion in health contains hydrochloric acid, combined hydrochloric acid, pepsin, rennin, acid salts and in diseased conditions, organic acids in addition. In health the stomach has a small amount of gastric juice constantly present and when food enters, the amount is greatly increased.

The object of the test meal is to determine the acidity of the secretion and the time required to reach the peak and its decline.

Instruct the patient the day before to come the following morning without breakfast. In order to make a perfect test, remove some of the stomach fluid prior to the administration of the meal. Examine and place on the chart under the heading of **fasting stomach**, which is abbreviated "F. S."

In order to obtain the F. S. acidity, it is necessary to pass the tube and also remove it before eating the meal. If trouble is encountered in passing the tube it may interfere with digestion to such an extent as to destroy the accuracy of the test. As a rule the tube is passed only after the meal and as a consequence no F. S. acidity can be obtained.

The meal quite generally used is 400 c.c. of corn flakes and 400 c.c. of water. The flakes must be thoroughly masticated during the time allowance of at least fifteen minutes. Have the patient drink

part of the water during the meal, but only when no food is in the mouth. Reserve part of the water to drink at the time the tube is inserted.

The best method of passing the tube is to place the olive tip back in the throat as far as consistent and have the patient close his lips and swallow several times in rapid succession. Push a little more of the tube into the throat of the patient and repeat the swallowing process. If in doubt whether or not the tube is in the stomach, force a little air down the tube by means of a syringe. If a gurgling sound is heard the tube is in the stomach contents.

There are two methods of removing the test meal. One is with a large tube passed into the stomach one hour after the beginning of the meal, removing as much of the contents as possible. The other is the fractional method which is most generally used. The tube, as designed by Rehfuss, is a small rubber tube with a metal olive tip which sinks to the bottom of the stomach. It should be passed after completing the meal and remain during the entire procedure.

Each fifteen or thirty minutes, as instructed, remove a small quantity, at least 5 c.c. of the stomach contents by means of suction, using a 20 c.c. glass syringe. It is advisable to pass a little air down the tube following the removal of the specimen in order to blow out the food liable to lodge in the olive tip which would swell and clog the inlet. Continue, at the designated intervals, to extract the stomach fluid until the stomach is empty or the gastric acidity has reverted to that of the fasting stomach.

Filter the contents removed through gauze as filter paper may absorb too much of the fluid, especially if there is only a small amount.

Total acidity consists of free hydrochloric, combined hydrochloric, organic acids and acid salts. The average is about 50 degrees.

The end point in this test is carried further than when testing for urine acidity, in order to neutralize the acid salts.

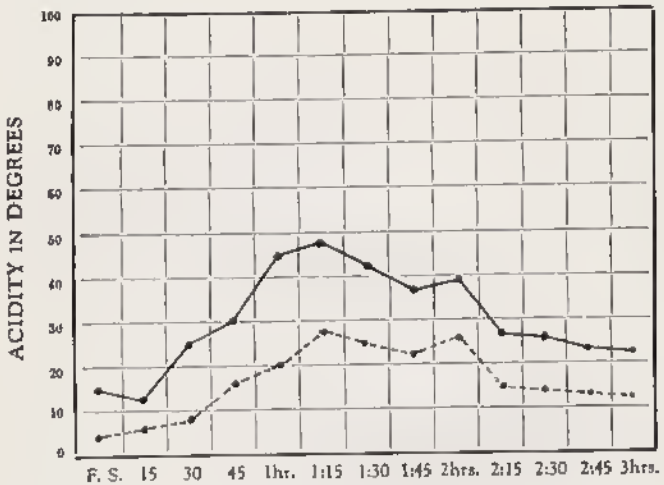

Fig. 16 — Diagram of Average Gastric Acidity: Continuous Line, Total Acidity: Dotted Line, Free Hydrochloric Acid

Toepfer's Method for Total Acidity

(a) Place 5 c.c. filtered stomach fluid in a 50 c.c. beaker.
(b) Add about 20 c.c. distilled water.
(c) Add 3 drops phenolphthalein solution.

(d) Titrate with N/20 sodium hydroxide until a distinct pink persists after stirring.
(e) Multiply the number of cubic centimeters sodium hydroxide used by 10 for the degrees total acidity.

Free hydrochloric acid ranges in normal stomachs from 20 to 30 degrees at the height of digestion. It activates pepsinogen which changes into pepsin, and loosely unites with protein, forming the combined acid. Hydrochloric is also a disinfectant. An increased amount of hydrochloric indicates neurosis, ulcer, or beginning of chronic gastritis. It may be due to chronic appendicitis or gall-bladder infection. A decreased amount of hydrochloric may be due to neurosis, gastric cancer, or chronic gastritis.

Toepfer's Method for Hydrochloric

(a) Place 5 c.c. of filtered stomach fluid in a 50 c.c. beaker.
(b) Add about 20 c.c. distilled water.
(c) Add 5 drops of the dimethylaminoazobenzol solution.
(d) Titrate with N/20 sodium hydroxide until a lemon yellow color appears. If hydrochloric is absent, the yellow color appears immediately.
(e) Multiply the cubic centimeters sodium hydroxide used by 10 for the degrees of hydrochloric.

The total acidity and free hydrochloric may be run on the same 5 c.c. of stomach fluid, by adding the two indicators at the same time. They will work entirely independently. Suppose 3.4 c.c. sodium hydroxide was required to obtain the yellow color, and an additional 2 c.c. to obtain the pink color: the free hydrochloric would be calculated on the basis of the 3.4 c.c. and the total acidity on the 5.4 c.c., or total volume hydroxide used.

Combined acid ranges from 10 to 15 degrees and represents the hydrochloric which has combined with the protein. The combination being very loose gives a positive acid reaction and is the first stage of protein digestion. It is always present when free hydrochloric is found and need not be routinely run. If hydrochloric is absent or present in very small quantities, it is best to run the combined acids. The amount of combined acids plus the amount of free hydrochloric determines the total amount of hydrochloric secreted.

Fig. 17 — Rehfuss Tube

Since there is no known indicator which determines the degrees of combined acid directly, it is necessary to obtain it by the indirect method, which gives all the degrees acidity except combined. Subtracting this amount from the total acidity gives the degrees of combined acid.

.Toepfer's Method for Combined Acid

(a) Place 5 c.c. filtered stomach fluid in a 50 c.c. beaker.
(b) Add about 20 c.c. distilled water.
(c) Add 5 drops of the sodium alizarin sulphonate solution.
(d) Titrate with N/20 sodium hydroxide until a violet color appears.

(e) Multiply the cubic centimeters sodium hydroxide used by 10 and subtract from the degrees total acidity, which gives the degrees combined acid.

Organic acids are the result of fermentation and consist of lactic, acetic and butyric. They are usually present when hydrochloric is deficient or absent, or when there is a low motility of the stomach. They occur in chronic gastritis and gastric cancer. Lactic acid is the one for which the test is usually made.

Kelling's Test for Lactic Acid

(a) Take two test tubes exactly alike.
(b) Fill one with distilled water.
(c) Add 10% ferric chloride until a light straw color is seen when looking straight down through the tube.
(d) Divide the solution equally between the two tubes.
(e) Add a small amount of the filtered gastric fluid to one tube.
(f) If lactic acid is present, the mixture will be a deeper yellow.

Pepsin is a ferment found in the gastric juice. It is secreted in the form of pepsinogen, having no power to digest until activated by hydrochloric acid which changes it into pepsin. It is seldom if ever absent when free hydrochloric is present.

Test for Pepsin

(a) Place 10 c.c. filtered stomach fluid in one of two similar test tubes.
(b) If previous tests have showed hydrochloric acid deficient, add 3 or 4 drops of the dilute acid.
(c) Place 10 c.c. water in a second test tube, add 3 or 4 drops dilute hydrochloric acid and 0.3 gm. pepsin.
(d) Place a piece of coagulated albumin about the size of a match head in each tube.

(e) Place both tubes in a water bath at about 37°C. for 3 hours.

(f) If digestion has taken place in both tubes pepsin is present in the gastric juice.

Blood when found in the stomach contents means disease or is a result of an injury due to passing the tube. If there is only a small amount and bright red in color, it is probably due to a mechanical injury. If found in any quantity and dark red, it usually indicates an ulcer, cancer or an aggravated case of chronic gastritis. The best method of testing for blood is the benzidine test. Microscopic examinations are unsatisfactory, as the gastric juice distorts the shape of the blood cells, making them difficult to recognize.

Make the test for blood the same as for blood in the urine, with the exception that the gastric fluid is not boiled.

Pus rarely is found in the stomach fluid and when present indicates an aggravated condition, in all probability cancer.

Mucus is found in very small quantities caused by the irritation of the tube. When an excess amount is found or of a dark color, it indicates a chronic inflammatory condition.

Occasionally remnants of food from a previous meal will be found. This is suggestive of slow motility. Sometimes particles of tissue are removed during the test, indicating malignancy.

The only germ of any consequence is the **Boas-Oppler Bacillus**, which is invariably found in cancerous conditions. It resembles bacillus acidophillus and takes a Gram stain.

CHAPTER V

HEMATOLOGY

Enumeration of Cells

Blood is composed of red cells, white cells and platelets which float in a liquid called plasma.

The red cells are called **erythrocytes.** They are bi-concave and non-nucleated. When a large number are grouped together they have a red appearance, but when seen in small groups they have a yellowish cast. The coloring matter of the erythrocytes is called **hemoglobin.**

In considering the red cells the two main objects of importance to the physician are the percentage of hemoglobin and the number of red cells per cubic millimeter. To find them one drop of blood is usually all that is necessary.

In health there are approximately 5,000,000 red corpuscles per cubic millimeter in men and 4,500,000 in women. To determine the number requires a very accurate technique. Because of the large number of red cells dilute the blood before attempting to make the count. Use a 100 or 200 dilution, the latter is preferred. The 100 dilution is used only in cases of anemia where the number of cells is greatly reduced.

For counting erythrocytes, use a special capillary pipette enlarged into a bulb near its upper end. Just above the bulb is the mark 101, and two marks, 0.5 and 1, are below.

A **hemacytometer,** the instrument used in counting blood, consists of three small platforms, a central and two lateral. The laterals are exactly 0.1 mm.

higher than the central or island one, so when a special cover slip is placed over them a chamber 0.1 mm. in depth is formed. Have the cover slip in perfect contact with the lateral platforms. When viewed obliquely color rings, know as Newton's bands, are visible.

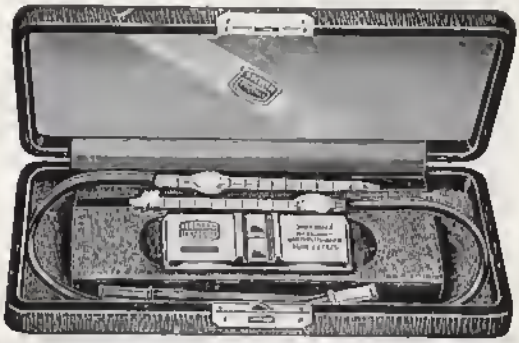

Fig. 18 Bright Line Hemacytometer in Case

On the inner platform or island of the hemacytometer nine square mms. are marked. The central one is subdivided into 400 smaller squares, each representing 1/400 of a square mm. If all the cells in one square mm. are counted, multiply by 10 which gives the number in one cubic mm. of diluted blood. This amount multiplied by 200 gives the number of cells in undiluted blood.

Very few technicians take the time to count all the cells in 400 small squares. Count the cells in a given number of squares and divide by the number of squares counted to obtain the average. This number multiplied by 400 gives the approximate number of cells per square millimeter.

There is a shorter method in general use which is simple yet accurate. Count sixteen squares in each of the four corners and the center of a square mm. which makes a total of eighty small squares. To this number of cells add four ciphers to obtain the number of cells in a cubic millimeter of undiluted blood. Modern hemacytometeres are marked so the sixteen squares are easily discernible. If an old type of hemacytometer is used time can be saved by the following rule: Count the number of cells in twenty-five small squares in each of the four corners of a square mm., multiply the total by eight and add three ciphers.

Unless a person is expert in counting, fill the counting chamber twice and make two individual counts to check for accuracy.

Technique for Counting Red Cells

(a) Cleanse and disinfect the end of a finger or the lobe of an ear.

(b) Puncture with a sterile lance or hypodermic needle.

(c) Wipe off the first drop of blood. If none appears, puncture again. Never massage the part.

(d) For the 200 dilution draw the blood to the 0.5 mark. Hold the pipette in a horizontal position, nearly at right angles to the line of vision, so the column of blood is constantly under observation. Have the tip of the pipette in the blood but not against the skin. Be sure that no air bubbles enter the pipette. Wipe the end of the pipette to remove all blood on the outside of tip.

(e) Draw diluting fluid, Toisson's or Hayem's, to the 101 mark and shake for a minute or two with up-and-down motion, never lengthwise.

(f) Blow out two or three drops on a little cotton or blotting paper to empty the capillary portion of the pipette.

(g) Place cover slip in perfect contact with lateral platforms of hemacytometer.

(h) Hold the tip of the pipette against the edge of the cover slip, between it and the floor of the Island. The blood will be drawn into the chamber by capillary attraction. Be sure that enough blood enters the chamber to completely cover it, but not enough to raise the cover slip as the count will be inaccurate.

(i) Use the low or the high dry objective of the microscope. Move the chamber until the little squares are visible.

(j) Count sixteen squares in each of the four corners and the center, which makes a total of eighty. Then add four ciphers to find the number of cells in one cu. mm. of undiluted blood.

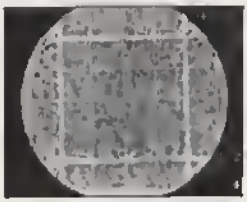

FIG. 19 — HEMACYTOMETER FIELD VIEWED UNDER MICROSCOPE

Under normal conditions the leukocytes vary from 5,000 to 10,000 with 7,500 as an average. If the number is temporarily over 10,000 there is a condition known as leukocytosis, which usually indicates some infection. In case the number is permanently over 10,000 the condition is known as leukemia.

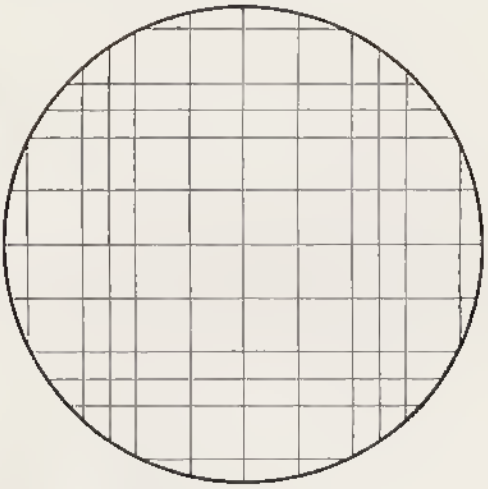

FIG. 20.—OLD HEMACYTOMETER RULING (Magnified)

Technique for Counting White Cells

(a) Cleanse, disinfect and puncture the same as for counting red cells.

(b) Dilute the same as for red cells with the exception that a 1-20 dilution is made. Draw the blood to the 0.5 mark in a special pipette, then the diluting fluid, which is usually 1 or 2 per cent acetic acid, is drawn to the 11 mark.

(c) After thoroughly shaking, blow three or four drops out and place under cover slip of hemacytometer the same as for red cells.

(d) Count all the cells in enough square millimeters until you have at least 200 cells counted. Divide the number of cells counted by the number of squares used to obtain the average per square millimeter. Multiply by 10 which gives the number of white cells per cubic millimeter of diluted blood. Now multiply by 20 to get the number for the undiluted blood.

Blood-platelets are colorless round bodies varying in size from one-fourth to one-half the diameter of an erythrocyte. They are constantly changing in number but average about 300,000 per cubic millimeter when counted by the direct method. Do not place too much dependence upon a single count but take the average of several counts made on different days.

Technique for Counting Blood-Platelets

(a) Cleanse, disinfect and puncture the same as for red cells.

(b) Use a red cell pipette and draw the diluting fluid, Leake and Guy, to the 1 mark, then the blood to the 0.5 mark and again the diluting fluid to the 101 mark, making a 1-200 dilution.

(c) Thoroughly mix and immediately place in counting chamber.

(d) Wait ten minutes for the platelets to settle, count and calculate the same as for red cells.

After completing any of the above counts blow the remaining blood from the pipette, thoroughly wash with water, alcohol and ether. To completely dry the tube after washing, use a suction apparatus drawing air through it. Wash the cover slip and hemacytometer by using a soft cloth moistened with alcohol.

CHAPTER VI

HEMATOLOGY

Hemoglobin and Coagulation

Hemoglobin is the iron-containing protein of the blood. It is found only in the red cells except in certain diseased conditions. Hemoglobin varies in amount from 13.5 to 17.5 grams per 100 c.c. of blood. Different instrument manufacturers use different amounts of hemoglobin as their normal standard. As a consequence the percentage of the hemoglobin determined by one instrument will be different from the percentage taken from another unless the same normal standard is used.

To convert the percentage of hemoglobin into the actual amount, multiply the amount of hemoglobin used as the standard by the percentage found.

Example: The hemoglobin reading is 90%. The standard used by the manufacturer is 17 grams per 100 c.c. blood. Then 0.90x17 equals 15.3 grams per 100 c.c. blood.

Three methods for determining the percentage of hemoglobin will be described: Dare, Sahli, and Tallqvist.

The **Dare** method is very accurate. The instrument is simple and easy to operate. A revolving color scale and a specially prepared pipette are the main working parts. The former varies in color from a low to high intensity of shade. The pipette consists of two small pieces of glass, one clear and the other a milky color, clamped together. It is constructed so the two sides approximate each other except at

the distal end where a small uniform space lies between them. The instrument has two scales, one gives the hemoglobin in grams per 100 c.c. and the other in percentage using 16 grams as its standard.

Dare's Technique

(a) Thoroughly cleanse and puncture a finger or lobe of an ear.
(b) Draw the undiluted blood into the slit in the pipette.
(c) Place pipette in the hemoglobinometer, milky side out.
(d) Look through the electrically illuminated telescope, having the room relatively dark.
(e) Rotate the color ring until its shade exactly matches the blood.
(f) Read the hemoglobin percentage as indicated on the scale.

FIG. 21 — DARE'S HEMOGLOBINOMETER

The **Sahli** hemometer has a tube for diluted blood and one or two permanent standard color tubes. The principle used is based on the fact that in blood mixed with hydrochloric acid, the hemoglobin is changed to

FIG. 22.—SAHLI'S HEMOMETER

acid hematin. The Sahli method works well with either normal or abnormal hemoglobin. It gives a slightly lower reading that the Dare, as it uses 17 grams as its standard.

Sahli's Technique

(a) Place N/10 hydrochloric acid in the special tube to the 10 mark on the percentage side of the tube.
(b) Thoroughly cleanse and puncture a finger or lobe of an ear.
(c) Draw blood up to the 20 cu. mm. mark in the special pipette supplied.
(d) Wipe the pipette and blow the blood into the hydrochloric acid.
(e) Rinse the pipette several times by drawing the blood up and blowing it out.
(f) Allow to stand several minutes.
(g) Dilute with water, mixing well, until the color exactly matches the standard.
(h) Read the percentage at the bottom of the meniscus at the top of the fluid in the tube.

The **Tallqvist** is an easy method to use, but only relatively accurate. A little book containing small sheets of absorbent paper and a color scale is used. The colors range from 10 to 100, based on 14 gm. hemoglobin per 100 c.c. The Tallqvist gives a higher reading than the Dare. The method is ideal for bedside work.

Tallqvist Technique

(a) Thoroughly cleanse and puncture a finger or lobe of an ear.
(b) Allow a drop of the blood to be absorbed by a square of the paper.
(c) After drying a moment, put the specimen under the color scale to determine the percentage.

Color Index

The color index represents the amount of hemoglobin in each individual cell. It is determined by dividing the percentage of hemoglobin by the percentage of the red cells found using 5,000,000 as the

normal. Obtain the percentage of hemoglobin by either of the methods previously described. The percentage of red cells is easily and quickly ascertained by multiplying the first two figures of the red cell count by two. For example if the count is 4,500,000 the per cent would be 45 times 2 or 90. If the hemoglobin is 100 per cent and the red cells 100 per cent the color index would be 100 divided by 100 or 1 which is normal. If the hemoglobin is 50 per cent and the red cell count is 50 per cent the color index would be 50 divided by 50 which also is 1. Thus if the hemoglobin and per cent of cells diminish equally the color index remains constant. If the hemoglobin is 100 per cent and the red cell count 50 per cent the color index would be 100 divided by 50 or 2. Again if the hemoglobin is 50 per cent and the red cell count 100 per cent the index would be 50 divided by 100 or 0.5.

Coagulating Time

When blood stands it coagulates due to the formation of fibrin which entangles the cellular elements and produces a clot. The length of time necessary for this to form is called the coagulating time. This information is very valuable before certain operations to detect "free bleeders." The following simple test is used to determine the time necessary for the formation of a clot.

Test for Blood-clotting Time

(a) Thoroughly cleanse and puncture a finger or lobe of an ear.
(b) Place a drop of blood on a microscopic slide.
(c) Draw a needle through it once a minute.
(d) Count the minutes until shreds of fibrin begin to follow the needle.

(e) The number of minutes from the time the blood is taken until the formation of the fibrin determines the clotting time, which is normally four and a half minutes.

Bleeding Time

This is the time required for a small cut to stop bleeding. Do not confuse with coagulation time. It is much shorter in duration because of the juices from the tissues and the power of the skin to close up the wound. Usually from one to three minutes are required for the bleeding to stop in a small wound.

FIG. 23 — BLOOD BLOTS DEMONSTRATING BLEEDING TIME

Duke's Method

(a) Thoroughly cleanse and puncture the lobe of the ear with a rather large hypodermic needle.

(b) Use a piece of filter paper to absorb all the blood exuded each thirty seconds. The spots should diminish in size.

(c) Between the second and sixth blotting they should disappear entirely. If more than six blottings are obtained the bleeding is longer than normal.

CHAPTER VII

HEMATOLOGY

Differential Count

In order to determine the percentage of the various leukocytes, and abnormal red cells, it is necessary to make a carefully prepared well stained smear. Use two cover slips or two slides thoroughly cleaned and dried. When using the cover glasses touch one to the drop of blood on the end of the finger, making no direct contact with the skin. Remove and immediately place this glass, blood side down, upon the other cover glass. Press the two together with gentle pressure, then slide them apart rather quickly. If properly done a thin layer of blood, evenly distributed, will be on each glass.

The slide method for making the smear is most generally used and probably the most accurate. Place a drop of blood on the slide near one end, being careful to make no skin contact. Hold the slide horizontally with the blood side up. In the other hand hold another slide, placing the end on the first slide at an angle of 35 degrees and carefully move toward the blood until contact is made. The blood quickly spreads, filling the angle between the two slides. Steadily and slowly move the slide in the opposite direction and the blood will follow leaving a thin smear. Never push the blood in front of the slide if a thin smear is desired as the cells may be injured.

After making the smear, "fix" the slide or cover glass whichever is used. Probably the best method is to pass it high over a flame for a few minutes.

After the slide has been fixed, stain it. If Wright's stain is used the fixing and staining are done at the same time.

For many years Wright's stain has been quite generally used for differential blood counting, but the stain changes upon standing, being unstable, and therefore should be made up fresh at intervals of a

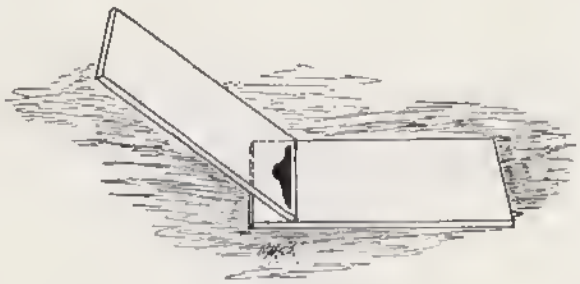

FIG. 24 — MAKING BLOOD SMEAR

few months.

Wright's Staining Method

(a) Cover the unfixed smear (counting the drops) with Wright's stain.

(b) Let stand one minute or longer as necessary to obtain the proper depth of color.

(c) Add the same volume of distilled water as the stain used. It is better, however, to use phosphate buffer solution, pH 6.4, instead of water.

(d) Allow to stand three to six minutes as necessary for proper differentiation.

(e) Flood with distilled water for thirty seconds, or until the film takes on a pinkish appearance.

(f) Dry by waving the slide over a flame, or by blotting.

Azure-II Eosin Staining

(a) Fix the smear by carefully heating over a flame for about forty-five seconds.
(b) Cover the smear (counting the drops) with the azure-II eosin stain for three minutes.
(c) Add the same volume of distilled water, and leave on for one minute, or less as necessary.
(d) Wash off excess stain with distilled water.
(e) Dry immediately over a flame.

Examine the slide by means of the oil immersion lens carefully focused. The picture presented to the analyst is very interesting. Hundreds of erythrocytes are seen with occasional leukocytes sprinkled through the field. There are two general divisions of leukocytes, normal and abnormal.

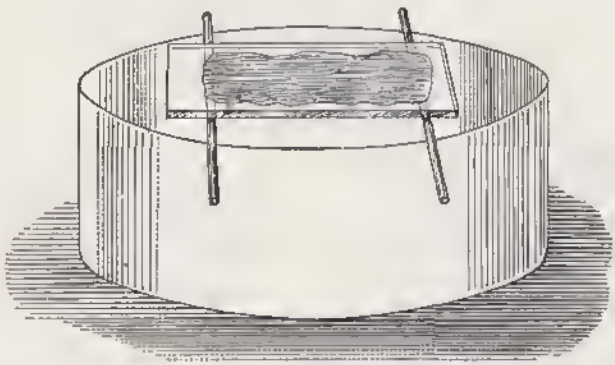

FIG. 25 — UNIQUE METHOD OF HOLDING SLIDE WHILE STAINING

The **normal type** can be divided into three general divisions—lymphocytes, monocytes and polymorphonuclear leukocytes.

Lymphocytes are always mononuclear. The magnitude of the nucleus in proportion to the size of the cell is their distinguishing feature. The amount of protoplasm is relatively small forming a narrow ring around the nucleus. It contains no granules of any great diagnostic value. Lymphocytes may be divided into two divisions according to their size. The small ones constitute 15 to 25 per cent of all leukocytes and are similar to the red cells in size. The large ones usually constitute 4 to 8 per cent of all leukocytes, and are about twice the size of the erythrocytes. The small variety is the mature cell while the larger one is immature.

According to our present knowledge no advantage is gained by dividing the lymphocytes into the two divisions and many laboratories are discontinuing the process. Because of their lack of granules lymphocytes are sometimes called **agranulocytes.**

The nucleus of each type is usually round but occasionally flattened a little on one side. It stains a very deep blue while the cytoplasm takes only a pale blue.

FIG. 26 — MONOCYTES

Monocytes are the largest cells found in the normal blood. There are two varieties, the mononuclear and the transitional. Some authorities consider them to be different stages in the life history of the same cell.

The mononuclear variety is similar to the large lymphocyte and many times hard to differentiate. The characteristic differences are as follows: the nucleus is smaller in proportion to the size of the cell and flat or indented on one side. These constitute 1 to 3 per cent of all leukocytes. The other variety has a horseshoe shaped nucleus and is called a transitional leukocyte. They constitute 2 to 5 per cent of all leukocytes. In the above varieties the zone of protoplasm surrounding the nucleus is much wider than in the lymphocytes and contains very fine granules which are often difficult to distinguish.

Either type of monocytes is nearly three times the size of the erythrocytes. They are sometimes called **endotheliocytes.**

Polymorphonuclears are smaller than the monocytes, being about the size of large lymphocytes. They are characterized by the various shapes of their nuclei. Most of the cells appear to have two to five nuclei however on close inspection they are found to be connected.

Polymorphonuclear leukocytes are divided into three groups according to the reaction of their granules to the stain. Cells with small granules taking a light stain are called **neutrophils** and constitute 60 to 70 per cent of all leukocytes. When the granules take a pronounced red or eosin stain the cells are called **cosinophils**, making 2 to 4 per cent of all leukocytes. The cells whose granules take a purple or basic stain are called **basophils** and constitute about 0.5 per cent of all leukocytes.

Because of the peculiar reaction of the granules

to the stain this group is sometimes called **granulocytes.**

In making the differential count, the neutrophils are "Schillingized" by dividing into four groups. Beginning with the youngest, we have the **myelocytes, juveniles, stabs,** and **segmenters.** The tendency toward segmentation increases in the order named. About 65 per cent of the differential count should consist of the segmenters, and 4 per cent of the stabs. Authorities differ as to allowing even one per cent of juveniles in a normal count but there should never be any myelocytes.

FIG. 27 — GRANULOCYTES

The first three of the above named neutrophils are called **shift cells.** An increase of these cells, with a corresponding decrease of segmenters, constitutes a **left shift.** Normally the shift is toward the right. A left shift indicates increased toxicity.

The **abnormal forms** of leukocytes are myeloblasts, myelocytes, juveniles, lymphoblasts and a few atypical forms.

The **myeloblasts** are parents of the polymorphonuclears. They range between 15 and 16 microns in diameter. The cytoplasm forms a very narrow rim which stains blue with a diffused effect, being free from granules. The nucleus is round and sharply defined. It contains several nucleoli. Occasionally vacuoles are present in the cell. These cells are never found in a healthy blood stream.

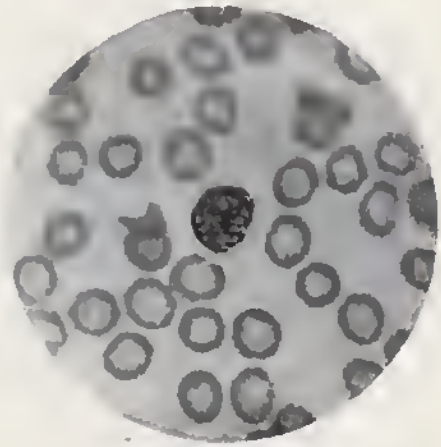

FIG. 28 — LYMPHOBLAST (Photo by Ziesel)

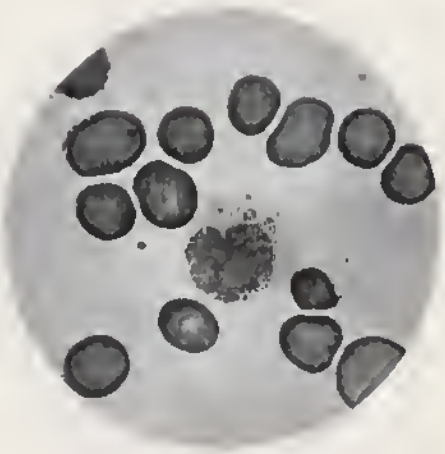

FIG. 29 — MYELOCYTE (Photo by Ziesel)

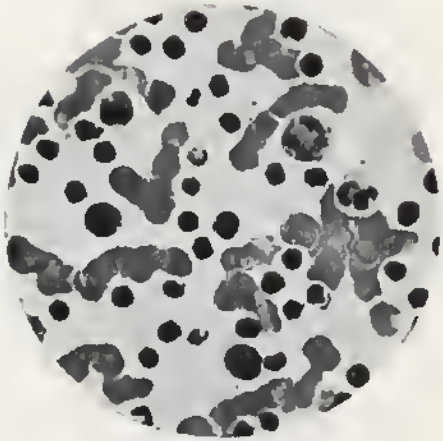

FIG. 30 — LYMPHOCYTES FROM A CASE OF LYMPHATIC LEUKEMIA
(Photograph by Ziesel)

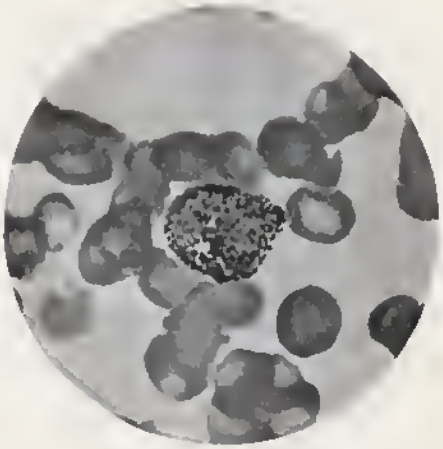

FIG. 31 — EOSINOPHIL WITH EXTRA LARGE GRANULES
(Photograph by Ziesel)

In the **myelocyte**, an abnormal form of leukocyte, the nucleus occupies about one-half the entire cell. It may be flat or slightly concave on one side. The myelocytes are direct descendants of the myeloblasts, about the same size but have granules.

The abnormal form of leukocytes most commonly seen is the **juvenile**. These cells often increase in number beyond the one per cent, sometimes normally allowed, when there is a left shift. The juveniles are differentiated from the myelocytes by their nuclei which are indented to practically 50% of their diameter.

The **lymphoblasts** are the parent cells of lymphocytes and never found in normal blood. They are larger than lymphocytes but in other respects, very similar. The cytoplasm forms a narrow band around a large nucleus and contains no granules. The nucleus contains from one to three nucleoli. The lymphoblasts are about the same size as the myeloblasts, making it difficult to differentiate between them.

In making a differential count, a mechanical stage is necessary in order to move the slide systematically. During the count, record each variety in a separate column. To assure accuracy, count a total of two hundred cells. Add each column separately, divide the sum by two, thus obtaining the number of each kind per hundred or the percentage.

Do not confine your count to any single portion of the slide but select different fields to make the count a fair average of the total cells. Schilling suggests dividing the slide into quadrants and to count one-fourth the total number of cells desired out of

each division. He also advises the "meander" method as illustrated below. Be sure to use the oil immersion lens to obtain an accurate count.

Much valuable information is obtained by a study of the differential count, especially by using Schilling's and Arneth's classifications.

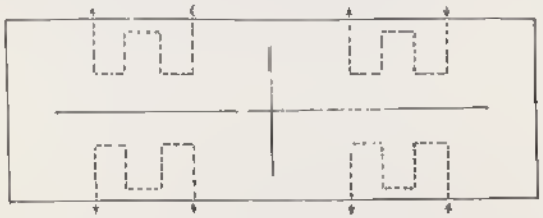

FIG. 32 — SCHILLING'S "MEANDER" METHOD FOR COUNTING LEUKOCYTES

Arneth divides polymorphonuclear neutrophils according to the number of segments in the nucleus. If the cells increase with a smaller number of segments it is considered a shift to the left, and if the cells increase with a larger number of segments it is a shift to the right. A shift to the left means infection.

Too much credit cannot be given to Schilling and Arneth for their valuable discoveries since many cases of serious infection have been detected before a sufficient rise in the total leukocyte count caused alarm.

Index of Resistance. A temporary rise in leukocytes above 10,000 is a condition known as leukocytosis, usually signifying a case of acute infection. The percentage of increase in the total number of leukocytes indicates the power of resistance the pa-

tient is manifesting toward the infection. The percentage of increase of polymorphonuclear neutrophils represents "the degree of toxic absorption." If the latter increase is proportionate to the total increase of leukocytes a good prognosis is presented. If the total increase is greater than the polymorphonuclear neutrophil increase the prognosis is excellent, but if the polymorphonuclear neutrophil increase is greater than the total increase the prognosis is grave. Wilson has given us a simple formula which gives an index of resistance. The formula is: (T—10)—(P—70) equals I. R. "T" represents the total leukocyte count expressed in thousands, "P" the per cent of polymorphonuclear neutrophils and "I. R." the index of resistance. When the resistance of the body and the degree of infection are approximately the same, the index of resistance would be zero.

During the differential count make a careful survey of the slide to ascertain if any abnormal red cells are present. Abnormal erythrocytes vary from the normal, in size, shape, structure and reaction to stain.

Cells smaller than normal are called **microcytes** and those larger than normal, **macrocytes**. In other respects they are normal. **Megalocytes** are larger than macrocytes, measuring from 12 to 18 microns.

Poikilocytes are abnormal as regards shape, having an oval or club form. If the blood has come in contact with water certain crenated forms are seen. Whether these ever exist in the blood stream is very doubtful.

Cells abnormal in structure are nearly always nucleated red cells called **erythroblasts**. If normal

in size they are called **normoblasts**, if smaller, **microblasts** and if larger, **macroblasts**. If a megalocyte has a nucleus it is a **megaloblast**. The erythroblast is supposed to lose its nucleus before entering the blood stream, thus becoming an erythrocyte. If the nucleus is not completely ejected with small particles remaining in the cell they are known as **Howell-Jolly** bodies. When only an outline of the nucleus is left it is called a **Cabot's ring**.

FIG. 33 — ERYTHROBLAST (Photo by Zeiss.)

Some erythrocytes are perfectly normal in size, shape and structure but react differently to stain.

A healthy red cell takes an acid stain giving it a yellowish-red tint. Some diseased cells take a basic stain which makes them blue or portions of them blue. In this instance the cell is a **polychromatophil**.

When there are irregularly shaped granules in the red cells giving a basic reaction they are called **basophilic erythrocytes**.

The polychromatophilic cells probably are young cells, while those containing basophilic granules are considered degenerative cells.

Vital Staining

Certain young erythrocytes possess a reticulum in their protoplasm and for this reason are called **reticulocytes**. Normally they constitute less than 1 per cent of the total red cells. In order to demonstrate them it is necessary to stain before molecular death takes place by a process called **vital staining**.

Schilling's Method for Vital Staining (Modified)

(a) Spread a very thin layer of the cresyl blue solution on a clean slide, free from fat.
(b) Allow to dry in the air.
(c) Spread a thin layer of blood over the dried dye in the same manner as for a differential count.
(d) Place immediately in a covered Petri dish which is kept damp by a piece of moist filter paper. Let remain 5 to 10 minutes.
(e) Remove and dry in the air.
(f) Counter stain with Wright's stain, wash and dry.

CHAPTER VIII

BLOOD TYPING

Until recently transfusion of blood was not attempted without considerable risk to the patient. In some cases there was no reaction while in others it was so great that death resulted.

In recent years experimentation has proved there are four types of blood and that two persons with the same type can give or take, but two persons with different types can neither give nor take without the possibility of danger. When two dissimilar types of blood come in contact with each other, agglutination takes place and in order to prevent this reaction the donor's blood must belong to the same type or group as the patient's.

In the process of agglutination there are two factors at work, one in the serum called agglutinin and the other in the corpuscles called agglutinogen. Upon further study two types of agglutinin were found, designated as a and b, and the corresponding two types of agglutinogen, designated as A and B.

Agglutinin and agglutinogen of the same type cause agglutination. Therefore if the blood contains both they must necessarily be of opposite types, otherwise the blood would be self-agglutinating.

A careful study of Group I reveals that its corpuscles contain both agglutinogens A and B, but its serum does not contain either of the agglutinins. Therefore it can be designated as group AB.

A study of Group II shows its corpuscles contain agglutinogen A only and its serum contains agglutinin b only. This group can be designated as Ab.

Group III contains agglutinogen B and agglutinin a only, and can be designated as Ba.

Group IV contains no agglutinogen but has both agglutinins and can be designated as ab.

The following diagram illustrates the reactions of the above groupings.

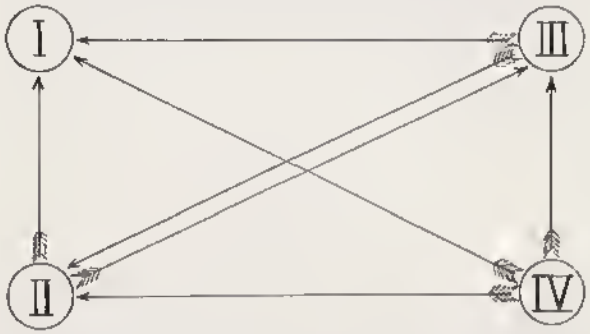

Fig. 34 — Sanford's Diagram Showing Interrelation of the Four Blood Groups

A careful survey of all four groups reveals that Group I can be agglutinated by any one of the other three, but in return will agglutinate none. Group IV is just the opposite. It will agglutinate any one of the other three and will not be agglutinated by any one of them. Group II will agglutinate Group III, and Group III will agglutinate Group II, thus producing a double agglutination.

The above groups are according to Moss. They differ from those of Jansky in that I and IV are

reversed. This has caused considerable confusion, as the hospitals use Moss' groups and scientists use Jansky's.

The naming of the groups by letters has simplified matters considerably. However, there is a move to further simplify it by using only the capital letters representing the agglutinogen, and zero where there is none. Therefore Group O Landsteiner would be Moss IV, or Jansky I; Group A Landsteiner would be group II, both Moss and Jansky; Group B Landsteiner is Group III, both Moss and Jansky; while Group AB Landsteiner is Moss I and Jansky IV.

Group O constitutes about 43 per cent of all cases, Group A, 40 per cent, Group B, 7 per cent, and Group AB, 10 per cent, respectively.

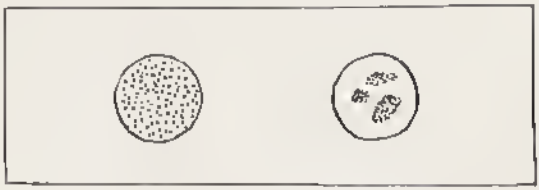

FIG. 35 — BLOOD MATCHING

Blood matching is a direct comparison of the donor's and recipient's blood. Testing only one person's blood to find in which group it belongs, is **blood typing**. All hospitals have several persons belonging to each group with their blood already typed, so in an emergency blood of the right type can be quickly obtained. Nevertheless, to avoid any possibility of mistake, match the donor's and patient's blood before attempting transfusion.

To match blood it is necessary to have a red-cell suspension and serum from both the donor and the recipient. To make the suspension use a "white" pipette, as in blood counting, with a 1 to 20 dilution of a 1% solution of sodium citrate in 0.8% saline. For the serum obtain a few drops of blood and let stand until it coagulates or centrifuge.

Place together on a slide the donor's serum and the recipient's cells and also the recipient's serum and the donor's cells. With the microscope ascertain if either combination agglutinates and if so the blood is unsafe for use.

In order to type an individual's blood, the simple test given by Vincent is sufficient. Place on a microscopic slide a drop of serum from a known case of Type II and another drop from a known case of Type III. Place in each a drop of the red-cell suspension or a small platinum loopful of blood taken directly from the pierced finger of the person whose blood is being typed. If agglutination takes place in both drops it belongs to Group I. If neither agglutinates it belongs in Group IV. If the unknown is agglutinated by II and not III it belongs to Group III, and if it is agglutinated by III but not by II it is Group II. (According to Moss.)

When it is impossible to obtain a donor with the right type of blood, Group IV may be used on any of the other three types in an emergency with only slight danger if injected slowly. The reason is that Group IV is not agglutinated by any of the other three groups, but will agglutinate, so by injecting slowly the dilution is so weak that there is very little harm done.

CHAPTER IX

BLOOD CHEMISTRY

The determination of the amount of non-protein nitrogen, urea, uric acid, creatinine, sugar, chlorides, phosphorus, and calcium present in the blood is termed blood chemistry. At the present time more clinical significance is being attached to it than in the past.

For an accurate test obtain the blood in the morning before the patient partakes of any food. Remove from the vein 5 to 10 c.c. of blood according to the number of tests required.

Technique for Obtaining the Blood:

(a) Cleanse the skin over the flexor surface of the elbow with soap and water.

(b) Disinfect with tincture of iodine and alcohol.

(c) Sterilize a 10 or 20 c.c. syringe and a 20 — 25 gauge needle.

(d) Apply a tourniquet (soft rubber tubing) above the elbow. Have patient extend arm and open and shut the hand a few times to cause the vein to stand out prominently.

(e) With the syringe parallel with the vein, insert the needle into the top of the vein or at either side.

(f) Hold the syringe steady and withdraw the required amount of blood.

(g) Remove the tourniquet before withdrawing the needle.

(h) Hold a square of sterile cotton, soaked in alcohol, firmly over the skin puncture to keep any blood from oozing out.

(i) Place the blood in a small bottle which contains about 2 mg. sodium oxalate for each cubic centimeter of blood used. Mix the blood with the oxalate to prevent clotting.

There are two types of nitrogen compounds present in the blood, **protein** and **non-protein**. In many pathological conditions the non-protein nitrogen is increased and its quantitative determination is advisable.

FIG. 36 — METHOD FOR OBTAINING BLOOD

The protein nitrogen must be removed before the non-protein nitrogen can be determined. The following method is very satisfactory.

Folin and Wu Method of Obtaining Blood Filtrate (Modified)

(a) Pipette the oxalated blood into a 200 c.c. flask. Call the cubic centimeters of blood used one volume.

(b) Add 6 volumes distilled water and mix.

(c) Add 1 volume 10% sodium tungstate.

(d) Add slowly, with constant agitation, 2/3 N. sulphuric acid (about one volume) until a chocolate brown color appears.

(e) Let stand 10 minutes. If the color is at all red like tomato soup, add more sulphuric. This requires experience.

(f) Add just enough distilled water to bring the total to exactly 10 volumes.
(g) Filter. The filtrate must be clear, if not, refilter.
(h) If the filtrate cannot be used at once, add a few drops of xylene and place in the ice box.
(i) 10 c.c. filtrate is considered equivalent to 1 c.c. blood.

The filtrate obtained after removing the blood protein contains all the waste nitrogen, sugar and mineral elements. The quantity of nitrogen in the several non-protein compounds may be determined separately if desired, or may be obtained collectively by the method of Folin and Wu. From 25 to 35 mg. of **non-protein nitrogen** is the amount normally present in 100 c.c. of blood.

Folin and Wu Method of Determining Non-protein Nitrogen

Standard	Unknown
(a) Place 2 c.c. acid digestive mixture in a 100 c.c. graduate.	(a) Place 1 c.c. acid digestive mixture in the NPN. tube.
(b) Add 3 c.c. standard ammonium sulphate solution (contains 0.3 mg. nitrogen)	(b) Add 5 c.c. filtrate and boil until solution is a clear straw color. Cool slightly.
(c) Add distilled water up to 70 c.c. mark.	(c) Add about 25 c.c. distilled water. When cold, add water to 35 c.c. mark.
Nesslerize both tubes at the same time	
(d) By adding 30 c.c. Nessler's reagent. Stopper and shake.	(d) By adding 15 c.c. Nessler's reagent. Stopper and shake.

(e) Compare immediately by means of colorimeter.
(f) Calculation: $\frac{\text{Standard}}{\text{Unknown}} \times 30$ equals mg. N.P.N. per 100 c.c. blood.

Creatinine is one of the nitrogen products included in the non-protein group. Many times it is advisable to determine the amount separately. When such is the case use the following technique, after Folin and Wu. The normal amount usually found in 100 c.c. of blood is 1 to 2 milligrams.

Determination of Blood Creatinine

Standard	Unknown
(a) Place 5 c.c. standard creatinine solution in a 50 c.c. graduate (contains 0.03 mg. creatinine)	(a) Place 10 c.c. filtrate in a large test tube.
(b) Add 15 c.c. distilled water.	(b) ————
(c) Add 10 c.c. alkaline picrate solution.	(c) Add 5 c.c. alkaline picrate solution.
(d) Shake.	(d) Shake.

Let stand eight minutes.

(e) Compare by means of a colorimeter.

(f) Calculation: $\frac{\text{Standard}}{\text{Unknown}} \times 1.5$ equals mg. creatinine per 100 c.c. blood.

Urea nitrogen constitutes about fifty per cent of the total non-protein nitrogen of the blood, ranging normally from 12 to 15 mg. per 100 c.c. The aeration method of Folin and Wu and the Karr nesslerization technique are described.

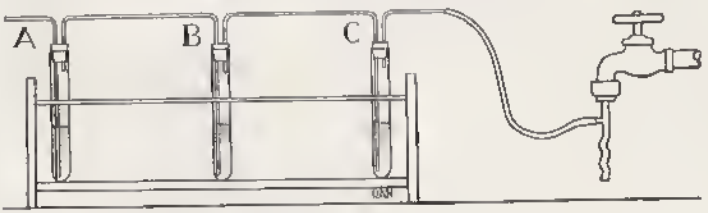

Fig. 37 — Diagram for Aeration Method

The Aeration Method

(a) Arrange three tubes, A, B, and C, as illustrated, tube C being nearest an aspirator pump, while A is at the other end of the series where air enters.

(b) Place about 15 c.c. 20% sulphuric acid in tube A, to remove the ammonia from the air.

(c) In tube C, place 1 c.c. N/10 hydrochloric acid and 15 c.c. distilled water to absorb the ammonia generated in tube B.

(d) In B, the middle tube, place 5 c.c. filtrate.

(e) Add to tube B 1 c.c. distilled water containing 50 mg. activated urease Jack bean meal.

(f) Place tube B in water bath at 50° C. for at least 5 minutes.

(g) Add 2 c.c. 10% sodium hydroxide and quickly cover the mixture with 2 c.c. amyl alcohol and immediately stopper.

(h) Aerate gently for 45 minutes.

(i) Label tube C, "Unknown", for the generated ammonia in tube B has been carried over into C.

Make the set up as follows:

Standard	Unknown
(a) Place 70 c.c. distilled water in a 100 c.c. graduate.	(a) ————
(b) Add 3 c.c. standard ammonium sulphate solution (contains 0.3 mg. nitrogen).	(b) ————
(c) Add 10 c.c. Nessler's reagent.	(c) Add 2.5 c.c. Nessler's reagent to tube C.
(d) Add distilled water up to 100 c.c.	(d) Add water up to 25 c.c.
(e) Shake.	(e) Shake.

(f) Read immediately in colorimeter.

(g) Calculation: $\frac{\text{Standard}}{\text{Unknown}} \times 15$ equals mg. urea per 100 c.c. blood.

The Folin and Wu method of urea nitrogen determination is often unsatisfactory since there are sources of error due to faulty aeration. The Karr direct nesslerization method is free from the above inaccuracies and should be the one routinely used.

The Karr Method (Modified)

Standard	Unknown
(a) Place 1 c.c. standard ammonium sulphate solution (contains 0.07 mg. nitrogen) in a 50 c.c. flask.	(a) Place 5 c.c. filtrate in a 50 c.c. flask.
(b) Add 4 c.c. distilled water	(b) ————
(c) Add 5 drops urease solution.	(c) Add 5 drops urease solution.
(d) Add 5 drops Karr's buffer solution.	(d) Add 5 drops of the buffer solution.
(e) Place in water bath at 55° C. for at least 30 minutes.	(e) Place in water bath at 55° C. for 30 minutes.
(f) Add 1 drop 2% gum ghatti solution.	(f) Add 1 drop 2% gum ghatti solution.
(g) Add 20 c.c. distilled water.	(g) Add 20 c.c. distilled water.
(h) Mix.	(h) Mix.
(i) Add 3 c.c. Nessler's reagent.	(i) Add 3 c.c. Nessler's reagent.

(j) Place in the colorimeter and read immediately.

(k) Calculation: $\frac{\text{Standard}}{\text{Unknown}} \times 14$ equals urea nitrogen per 100 c.c. blood.

Uric acid also is one of the non-protein compounds found in the blood. The normal amount ranges from 2 to 4 milligrams per 100 c.c. For its determination the Benedict method is preferred.

Determination of Blood Uric Acid

Standard	Unknown
(a) Place 5 c.c. standard uric acid solution in a large test tube (contains 0.02 mg. uric acid).	(a) Place 5 c.c. filtrate in a large test tube.
(b) Add 5 c.c. distilled water.	(b) Add 5 c.c. distilled water.
(c) Add 4 c.c. 5% sodium cyanide solution.	(c) Add 4 c.c. 5% sodium cyanide solution.
(d) Add 1 c.c. uric acid reagent.	(d) Add 1 c.c. uric acid reagent.
(e) Shake gently.	(e) Shake gently.

(f) Immerse both tubes in boiling water for 3 minutes. Place both tubes in cold water for 3 minutes.

(g) Compare by means of a colorimeter.

(h) Calculation: $\frac{\text{Standard}}{\text{Unknown}} \times 4$ equals mg. uric acid per 100 c.c. blood.

The **chlorides** of the blood occur most frequently in the form of sodium chloride. This salt constitutes about 0.5 per cent of the entire blood, the largest amount of any single inorganic element. The amount increases in certain diseases and decreases in others. Nephritis has an increase while diabetes shows a marked decrease. Many times its determination is advisable.

Whitehorn's Method for Blood Chlorides (Modified)

(a) Place 10 c.c. filtrate in a 50 c.c. beaker.

(b) Add about 15 c.c. distilled water.

(c) Add 5 c.c. standard silver nitrate solution (1 c.c. equals 1 mg. sodium chloride).

(d) Add 5 c.c. concentrated nitric acid and mix, and let stand 5 minutes.

(e) Add 0.3 gm. ferric ammonium sulphate and shake.

(f) Titrate with potassium sulphocyanate solution (1 c.c. equals 1 c.c. silver nitrate solution) until a salmon red color persists a few seconds after mixing.

(g) Subtract the cub'c centimeters (calculated to hundredths) of potassium sulphocyanate used from 5 (the volume silver nitrate), and multiply this difference by 100.

(h) This result is the milligrams sodium chloride in 100 c.c. blood.

Sugar is normally found in the blood to the extent of about 1 milligram to 1 c.c. Its increase always indicates some pathological condition. The method advocated by Folin and Wu is recommended. For accurate results examine the specimen within two hours after removal.

Determination of Blood Sugar

Standard	Unknown
(a) Place 2 c.c. standard sugar solution in a sugar tube (contains 0.2 mg. dextrose).	(a) Place 2 c.c. filtrate in another sugar tube.
(b) Add 2 c.c. alkaline copper sulphate solution.	(b) Add 2 c.c. alkaline copper sulphate solution.

(c) Place both tubes in boiling water for 6 minutes. Cool 3 minutes.

(d) Add 2 c.c. molybdate phosphate solution.	(d) Add 2 c.c. molybdate phosphate solution.
(e) Add distilled water up to the 25 c.c. mark.	(e) Add distilled water up to the 25 c.c. mark.
(f) Mix.	(f) Mix.

(g) Compare by means of a colorimeter.

(h) Calculation: $\frac{\text{Standard}}{\text{Unknown}}$ x 100 equals mg. dextrose per 100 c.c. blood.

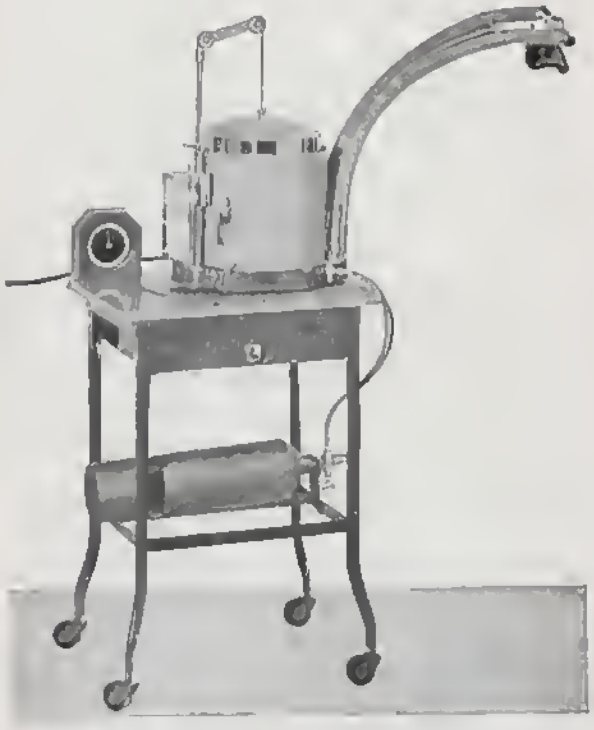

FIG. 38 — MODERN APPARATUS FOR TESTING METABOLISM

CHAPTER X

BASAL METABOLISM

Basal metabolism is a term used to designate the minimum heat production of the body. For an accurate determination the body must be in complete muscular relaxation and the test made after at least a 12 hour fast.

The basal metabolic rate is the amount of heat produced in one hour compared with the normal for the individual and is expressed in the percentage above or below normal. For example, +25 represents a metabolic rate of 25 per cent above normal, —25 a diminished rate of 25 per cent and from +10 to —10 is considered normal. The consumption of oxygen is controlled directly by the thyroid secretion therefore the metabolic rate gives valuable information concerning the activity of the gland. This is very essential for the proper diagnosis and treatment of certain disorders.

Basal metabolic rate can be ascertained directly by placing the patient in a calorimeter or indirectly by determining the amount of oxygen consumed during the hour. The latter is the method quite universally adopted.

The apparatus used must be scientifically designed and accurately built containing an oxygen chamber, a soda-lime chamber and a recording or measuring device. Some are provided with an electric blower which keeps the oxygen in circulation preventing the exhaled air from returning directly into the oxygen

tank. Others are supplied with valves to accomplish the same result.

The fundamental principle of all metabolism apparatus is the same: to deliver oxygen to the patient, record the actual amount consumed, and carry back to the oxygen compartment the exhaled air after the carbon dioxide has been removed by the soda-lime.

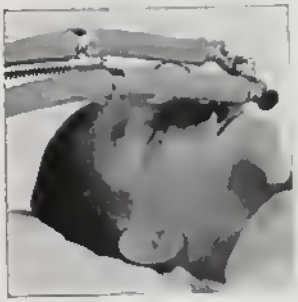

Fig. 39 — Application of Mouthpiece
Fig. 40 — Application of Mask

A thermometer to record the temperature of the oxygen used and a barometer to determine the barometric pressure of the atmosphere are necessary.

The recording device varies with different apparatus. The paper is ruled with horizontal and vertical lines. The distance between any two horizontal lines represents a definite quantity of oxygen while the distance between any two vertical lines represents a definite time. The drum is rotated by clockwork. A recent type is operated by an electric

clock which unwinds the paper at the rate of one inch a minute. A pen makes a graph on the paper, from which is determined the amount of oxygen consumed for a definite period of time.

Instructions to Patient

Do no fatiguing exercise or hard labor the day before.

Eat a light dinner not later than 8 o'clock in the evening and from then until bedtime do nothing of an exciting nature.

Retire early, never later than 10 o'clock thus getting a good night's rest of eight hours.

If restless during the night postpone the test.

In the morning, without breakfast, medicine or even a bath, come to the laboratory as soon after 8 o'clock as possible, never later than 9 o'clock.

Do not hurry and have someone drive the car for you.

Rest Period

Explain to the patient what you propose to do gaining his confidence from the start. His co-operation is necessary in order to obtain an accurate test.

Have patient go to rest room, after which remove all tight clothing, shoes and hair pins for complete relaxation. Place on a comfortable couch or bed with a soft pillow. The rest period is thirty minutes to one hour, depending upon the amount of energy consumed in reaching the laboratory. A well ventilated room with a temperature between 65 and 70 degrees Fahrenheit is necessary.

Do not converse or permit any conversation with the patient during the rest period and be sure that

no conversations or noises are within his hearing. He must not even read as complete relaxation is imperative.

Omit the rest period if the test is made in the patient's home and before he arises in the morning.

Technique

Prepare apparatus before the patient arrives or in an adjacent room. Thoroughly sterilize the mouthpiece and nose-clamp. Remove the tank and place the soda-lime in its proper chamber. Fill with water to the proper height the space provided for it. Replace the tank and fill with the required amount of oxygen, or air and oxygen combined, as suggested for each kind of apparatus. It is advisable to have an extra cylinder of oxygen to meet any emergency. Adjust the pen and fill with ink. Start the recording device to find if it operates perfectly.

Be sure there is no leak anywhere in the apparatus. This is determined by placing a cork in the end of the breathing tube, a small weight upon the tank and then starting the recording device. If there is no leak, the graph will be a straight horizontal line, otherwise, the line will have a tendency to rise. Give a final inspection to be sure everything is in perfect order to avoid any adjusting of the apparatus after it has been moved into the patient's room.

At the expiration of the rest period place the mouthpiece in perfect contact with the main part between the lips and gums and the two tips between the teeth. Place the nose-clamp so there is no breathing through the nose. Start the recording apparatus and have the patient breathe as normally as possible.

Allow it to run the desired number of minutes. Stop the apparatus, remove mouthpiece and nose-clamp and give the patient a rest of at least five minutes. Repeat the process. If there is any material difference between the two tests make a third one.

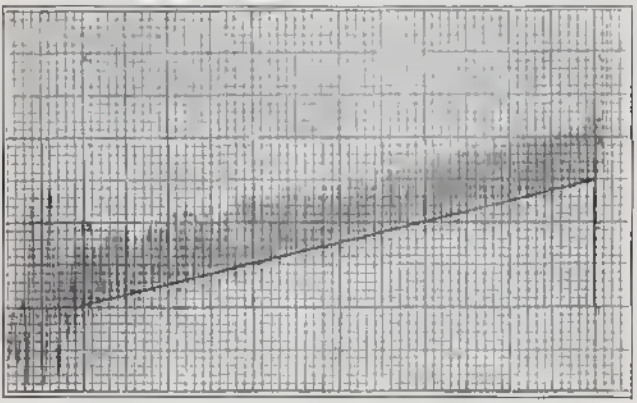

Fig. 41 — Graph Showing Amount of Oxygen Consumed

About the middle of the test take the temperature of the oxygen from the thermometer attached to the apparatus and also record the barometric pressure from a reliable barometer. Take the patient's temperature, pulse and respiration before or after the test. At the expiration of the test weigh and measure the patient. For accurate results weigh the patient with only a sheet around him and measure with shoes removed. Record the patient's age at his nearest birthday.

Calculations

In calculating the basal metabolic rate (B.M.R.) you must first ascertain the amount of oxygen consumed in one hour. This is accomplished by determining the actual amount in any given number of minutes and then for the hour. A six-minute period is generally used which is then multiplied by ten.

To obtain the amount of oxygen in a six-minute period draw a straight line parallel with and touching the lower points of the respiratory lines of the graph. If the graph covers more than a six-minute period select that portion which represents the best six minutes. The amount of oxygen is read by determining the number of horizontal lines bisected.

If there is little difference between the two graphs use the second one. If considerable difference and a third one is run use the average of the last two.

After determining the amount of oxygen consumed per hour proceed with the DuBois method which is divided into three steps.

The **first step** is to convert the amount of oxygen used per hour into calories by multiplying the number of liters used by the number of calories produced by one liter which under certain standard conditions is 4.825.

The number of calories per liter varies with the temperature and barometric pressure and the figure 4.825 would not apply in all conditions. Therefore it is necessary to change this amount to meet the varying conditions. Table A is so arranged that the exact figure for any given temperature and pressure can be obtained. Then multiply the number of liters of oxygen by this corrected figure and the result

is the actual number of calories under the existing temperature and pressure.

The chart of the Benedict-Roth apparatus is so arranged that it registers calories per hour instead of liters. Nevertheless this figure must be corrected for temperature and pressure. For this use Table D.

The **second step** is to determine the number of calories per square meter of skin area. First find the total surface of the body which requires two factors to be taken into consideration, the height and weight of the body.

DuBois has prepared a table which gives this information. Boothby and Sandiford of the Mayo Clinic have modified DuBois' chart. Column one shows the height both in feet and centimeters. Column two shows the weight in pounds and kilograms. Column three which is in the middle shows the surface area in square meters. See Table B.

To determine the skin area for a definite height and weight mark column one for the height, column two for the weight and with a ruler draw a straight line between these two points and read the body surface at the place where the line intersects the midline. Divide the number of calories obtained by the number of square meters of skin area and the actual number of calories per square meter of body surface of the individual tested is found.

The **third step** is to compare the number of calories per hour with the normal. DuBois has prepared a chart giving the normal per square meter of skin surface for both sexes. Boothby and Sandiford have modified the chart to range from 5 to 80 years of age. See Table C.

TABLE A

The caloric equivalent of one liter of Oxygen (4.825) corrected for pressure and temperature.

mm \	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
615	3.65	3.63	3.61	3.59	3.57	3.55	3.53	3.52	3.50	3.48	3.46	3.44	3.42	3.40	3.38	3.36	3.34	3.33	3.31	3.29	3.27	3.25	3.23	3.21
620	3.68	3.66	3.64	3.62	3.60	3.58	3.56	3.55	3.53	3.51	3.49	3.47	3.45	3.43	3.41	3.39	3.37	3.36	3.34	3.32	3.30	3.28	3.26	3.24
625	3.71	3.69	3.67	3.65	3.63	3.61	3.59	3.58	3.56	3.54	3.52	3.50	3.48	3.46	3.44	3.42	3.40	3.39	3.37	3.35	3.33	3.31	3.29	3.27
630	3.74	3.72	3.70	3.68	3.66	3.64	3.62	3.61	3.59	3.57	3.55	3.53	3.51	3.49	3.47	3.45	3.43	3.42	3.40	3.38	3.36	3.34	3.32	3.30
635	3.77	3.75	3.73	3.71	3.69	3.67	3.65	3.64	3.62	3.60	3.58	3.56	3.54	3.52	3.50	3.48	3.46	3.44	3.42	3.40	3.38	3.36	3.34	3.32
640	3.80	3.78	3.76	3.74	3.72	3.70	3.68	3.67	3.65	3.63	3.61	3.59	3.57	3.55	3.53	3.51	3.49	3.47	3.45	3.43	3.41	3.39	3.37	3.35
645	3.83	3.81	3.79	3.77	3.75	3.73	3.71	3.70	3.68	3.66	3.64	3.62	3.60	3.58	3.56	3.54	3.52	3.50	3.48	3.46	3.44	3.42	3.40	3.38
650	3.86	3.84	3.82	3.80	3.78	3.76	3.74	3.73	3.71	3.69	3.67	3.65	3.63	3.61	3.59	3.57	3.55	3.53	3.51	3.49	3.47	3.45	3.43	3.41
655	3.88	3.86	3.84	3.82	3.80	3.78	3.76	3.75	3.74	3.72	3.70	3.68	3.66	3.64	3.62	3.60	3.58	3.56	3.54	3.52	3.50	3.48	3.46	3.44
660	3.91	3.89	3.87	3.85	3.83	3.81	3.79	3.78	3.76	3.74	3.72	3.70	3.68	3.66	3.64	3.62	3.60	3.58	3.56	3.54	3.52	3.50	3.48	3.46
665	3.94	3.92	3.90	3.88	3.86	3.84	3.82	3.81	3.79	3.77	3.75	3.73	3.71	3.69	3.67	3.65	3.63	3.61	3.59	3.57	3.55	3.53	3.51	3.49
670	3.97	3.95	3.93	3.91	3.89	3.87	3.85	3.84	3.82	3.80	3.78	3.76	3.74	3.72	3.70	3.68	3.66	3.64	3.62	3.60	3.58	3.56	3.54	3.52
675	4.00	3.98	3.96	3.94	3.92	3.90	3.88	3.87	3.85	3.83	3.81	3.79	3.77	3.75	3.73	3.71	3.69	3.67	3.65	3.63	3.61	3.59	3.57	3.55
680	4.04	4.02	4.00	3.98	3.96	3.94	3.92	3.90	3.88	3.86	3.84	3.82	3.80	3.78	3.76	3.74	3.72	3.70	3.68	3.66	3.64	3.62	3.60	3.58
685	4.07	4.05	4.03	4.01	3.99	3.97	3.95	3.93	3.91	3.89	3.87	3.85	3.83	3.81	3.79	3.77	3.75	3.73	3.71	3.69	3.67	3.65	3.63	3.61
690	4.10	4.08	4.06	4.04	4.02	4.00	3.98	3.96	3.94	3.92	3.90	3.88	3.86	3.84	3.82	3.80	3.78	3.76	3.74	3.72	3.70	3.68	3.66	3.64
695	4.13	4.11	4.09	4.07	4.05	4.03	4.01	3.99	3.97	3.95	3.93	3.91	3.89	3.87	3.85	3.83	3.81	3.79	3.77	3.75	3.73	3.71	3.69	3.67
700	4.16	4.14	4.12	4.10	4.08	4.06	4.04	4.02	3.99	3.97	3.95	3.93	3.91	3.89	3.87	3.85	3.83	3.81	3.79	3.77	3.75	3.73	3.71	3.69
705	4.19	4.17	4.15	4.13	4.11	4.09	4.07	4.05	4.03	4.01	3.99	3.97	3.94	3.92	3.90	3.88	3.86	3.84	3.82	3.80	3.78	3.76	3.74	3.72
710	4.22	4.20	4.18	4.16	4.14	4.12	4.10	4.08	4.06	4.04	4.02	3.99	3.97	3.95	3.93	3.91	3.89	3.87	3.85	3.83	3.81	3.79	3.77	3.75
715	4.25	4.23	4.21	4.19	4.17	4.15	4.13	4.11	4.09	4.07	4.05	4.02	4.00	3.98	3.96	3.94	3.92	3.90	3.88	3.86	3.84	3.82	3.80	3.78
720	4.28	4.26	4.24	4.22	4.20	4.18	4.16	4.14	4.12	4.10	4.08	4.06	4.03	4.01	3.99	3.97	3.95	3.93	3.91	3.89	3.87	3.85	3.83	3.81
725	4.31	4.29	4.27	4.25	4.23	4.21	4.19	4.17	4.15	4.13	4.11	4.08	4.06	4.04	4.02	4.00	3.98	3.96	3.94	3.92	3.90	3.88	3.86	3.84
730	4.34	4.32	4.30	4.28	4.26	4.24	4.22	4.20	4.18	4.16	4.14	4.11	4.09	4.07	4.05	4.03	4.01	3.99	3.97	3.95	3.93	3.91	3.89	3.87
735	4.36	4.34	4.32	4.30	4.28	4.26	4.24	4.22	4.20	4.18	4.16	4.13	4.11	4.09	4.07	4.05	4.03	4.01	3.99	3.97	3.95	3.93	3.91	3.89
740	4.39	4.37	4.35	4.33	4.31	4.29	4.27	4.25	4.23	4.21	4.19	4.16	4.14	4.12	4.10	4.08	4.06	4.04	4.02	4.00	3.98	3.96	3.94	3.92
745	4.42	4.40	4.38	4.36	4.34	4.32	4.30	4.28	4.26	4.24	4.22	4.19	4.17	4.15	4.13	4.11	4.09	4.07	4.05	4.03	4.01	3.99	3.97	3.95
750	4.44	4.43	4.41	4.39	4.37	4.35	4.33	4.31	4.29	4.27	4.25	4.22	4.20	4.18	4.16	4.14	4.12	4.09	4.07	4.05	4.03	4.01	3.99	3.97
755	4.48	4.46	4.44	4.42	4.40	4.38	4.36	4.34	4.32	4.30	4.28	4.25	4.23	4.21	4.19	4.17	4.15	4.12	4.10	4.08	4.06	4.04	4.02	4.00
760	4.51	4.49	4.47	4.45	4.43	4.41	4.39	4.37	4.35	4.33	4.31	4.28	4.26	4.24	4.22	4.19	4.17	4.15	4.13	4.11	4.09	4.07	4.05	4.03
765	4.54	4.52	4.50	4.48	4.46	4.44	4.42	4.40	4.38	4.36	4.34	4.31	4.29	4.27	4.25	4.23	4.21	4.18	4.16	4.14	4.12	4.10	4.08	4.06
770	4.57	4.55	4.53	4.51	4.49	4.47	4.45	4.43	4.41	4.39	4.37	4.34	4.32	4.30	4.28	4.26	4.24	4.21	4.19	4.17	4.15	4.13	4.11	4.09
775	4.60	4.58	4.56	4.54	4.52	4.50	4.48	4.46	4.44	4.42	4.40	4.37	4.35	4.33	4.31	4.29	4.27	4.24	4.22	4.20	4.18	4.16	4.14	4.12
780	4.63	4.61	4.59	4.57	4.55	4.53	4.51	4.49	4.47	4.45	4.43	4.40	4.38	4.36	4.34	4.32	4.30	4.28	4.25	4.22	4.20	4.18	4.16	4.14

Find the normal from the chart. If smaller than the actual amount as determined above subtract normal from actual and ascertain the number of calories above normal.

If the normal is larger than the actual, subtract the latter to ascertain the amount less than normal.

In either instance divide the difference by normal to obtain the percentage above (+) or below (—).

Example: Patient, woman, age 32, weight 140 pounds, height 66 inches, temperature 98° F., consumes 12 liters of oxygen per hour, temperature of apparatus 25° C., barometric pressure 700 mm.

By referring to Table A the coefficient for the above temperature and pressure is 3.95; multiplied by the 12 liters gives 47.4 calories per hour. Using Table B the skin area is found to be 1.7. Divide the total calories (47.4) by the surface area of the body (1.7) and obtain 27.9 calories per square meter of skin area. The normal according to Table C is 36.2 which is more than the actual amount (27.9). Subtract the actual from the normal and we find the patient has 8.3 calories less than normal. Divide 8.3 by the normal (36.2) which gives 0.23 (23%) or —23 as the metabolic rate, with her subnormal temperature.

To calculate the rate if the patient's temperature were normal, 98.6° F., just remember that a fever of one degree increases the metabolic rate 7.2%, while a subnormal temperature of one degree would decrease the rate the same amount.

Since this patient has a subnormal temperature of 0.6° F., if her temperature were raised to normal, the metabolic rate would increase 4.32% (0.6 x 7.2) or raise from —23 to —18.68.

TABLE B

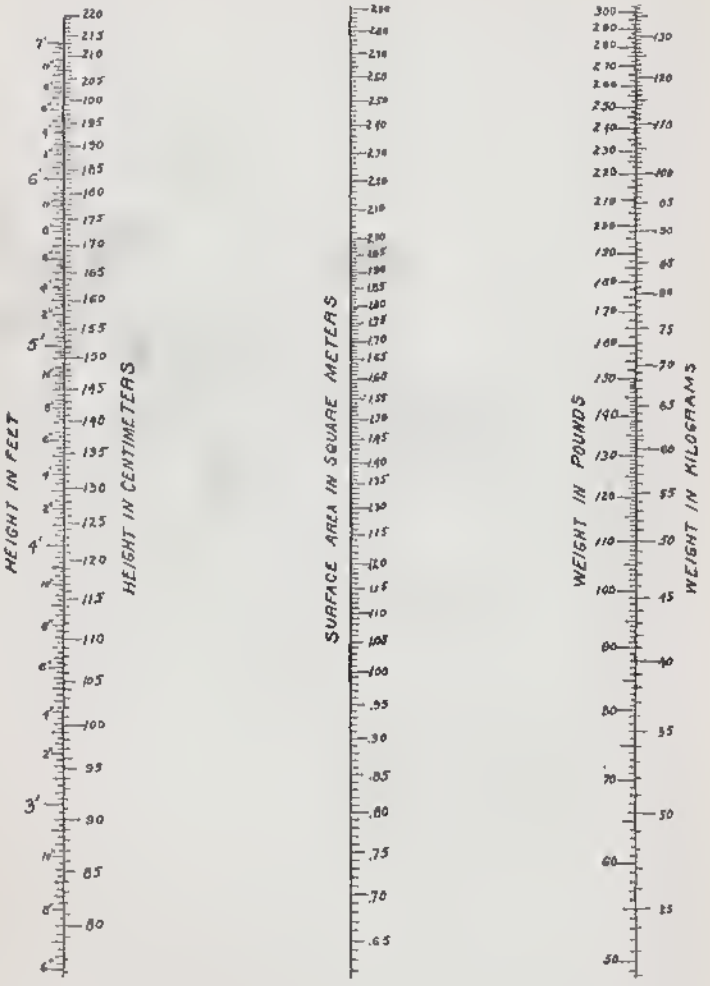

Chart for determining the Skin Area. Prepared by Boothby and Sandiford (Mayo Clinic) using the DuBois Standards.

TABLE C

THE DUBOIS NORMAL STANDARDS
Modified by Boothby and Sandiford (Mayo Clinic)
Calories per Square Meter per Hour

Age	Males	Females	Age	Males	Females
5	53.0	51.6	20-24	41.0	36.9
6	52.7	50.7	25-29	40.3	36.6
7	52.0	49.3			
8	51.2	48.1	30-34	39.8	36.2
9	50.4	46.9	35-39	39.2	35.8
10	49.5	45.8	40-44	38.3	35.3
11	48.6	44.6	45-49	37.8	35.0
12	47.8	43.4			
13	47.1	42.0	50-54	37.2	34.5
14	46.2	41.0	55-59	36.6	34.1
15	45.3	39.6	60-64	36.0	33.8
16	44.7	38.5	65-69	35.3	33.4
17	43.7	37.4			
18	42.9	37.3	70-74	34.8	32.8
19	42.1	37.2	75-79	34.2	32.3

TABLE D

Correction chart for use with the Benedict-Roth apparatus

mm	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
600	.735	.732	.728	.725	.721	.718	.711	.712	.708	.704	.701	.697	.693	.689	.685	.681	.677	.673	.669	.665	.661
601	.742	.739	.735	.731	.727	.724	.721	.718	.714	.710	.707	.703	.699	.695	.691	.687	.683	.679	.671	.671	.667
610	.748	.745	.741	.737	.733	.730	.727	.724	.720	.716	.713	.709	.705	.701	.697	.693	.689	.685	.680	.676	.672
615	.754	.751	.747	.744	.740	.737	.734	.730	.726	.722	.719	.715	.711	.707	.703	.699	.691	.691	.686	.682	.678
620	.760	.757	.753	.750	.746	.743	.740	.737	.733	.729	.725	.721	.717	.713	.709	.705	.701	.696	.692	.688	.684
625	.767	.764	.760	.756	.752	.749	.746	.743	.739	.735	.731	.727	.723	.719	.715	.711	.707	.702	.698	.694	.690
630	.773	.770	.766	.762	.758	.755	.752	.749	.745	.741	.737	.733	.729	.725	.721	.717	.713	.708	.704	.700	.696
635	.779	.776	.772	.768	.764	.761	.758	.755	.751	.747	.743	.739	.735	.731	.727	.723	.719	.714	.710	.706	.702
640	.785	.782	.778	.774	.770	.767	.764	.761	.757	.753	.749	.745	.741	.737	.733	.729	.725	.720	.716	.711	.707
645	.792	.788	.784	.781	.777	.773	.770	.767	.763	.759	.755	.751	.747	.743	.739	.735	.731	.726	.722	.717	.713
610	.798	.794	.791	.787	.784	.780	.777	.773	.769	.765	.761	.757	.753	.748	.744	.740	.736	.732	.727	.723	.719
655	.804	.800	.797	.793	.790	.786	.783	.779	.775	.771	.767	.763	.758	.754	.750	.746	.742	.738	.733	.729	.724
660	.810	.806	.803	.799	.796	.792	.789	.785	.781	.777	.773	.769	.764	.760	.756	.752	.748	.744	.739	.735	.730
665	.816	.812	.809	.805	.802	.798	.795	.791	.787	.783	.779	.775	.770	.766	.762	.758	.754	.749	.745	.741	.736
670	.822	.819	.816	.812	.809	.805	.801	.797	.793	.789	.785	.781	.776	.772	.768	.764	.760	.755	.751	.746	.742
675	.828	.825	.821	.818	.815	.811	.807	.803	.799	.795	.791	.787	.782	.778	.774	.770	.766	.761	.757	.752	.748
680	.834	.831	.827	.824	.821	.817	.813	.809	.805	.801	.797	.793	.788	.784	.780	.776	.771	.767	.763	.758	.754
685	.841	.837	.833	.830	.827	.823	.819	.815	.811	.807	.803	.799	.794	.790	.786	.782	.777	.773	.769	.764	.760
690	.848	.844	.841	.837	.833	.829	.825	.821	.817	.813	.809	.805	.800	.796	.792	.788	.783	.779	.771	.770	.765
695	.854	.850	.847	.843	.839	.835	.831	.827	.823	.819	.815	.811	.806	.802	.798	.794	.789	.781	.780	.776	.771
700	.860	.856	.853	.849	.845	.841	.837	.833	.829	.825	.821	.817	.812	.808	.804	.800	.795	.791	.786	.781	.777
705	.866	.862	.859	.855	.851	.847	.843	.839	.835	.831	.827	.823	.818	.814	.810	.806	.801	.796	.792	.787	.783
710	.872	.868	.865	.861	.857	.853	.849	.845	.841	.837	.833	.829	.824	.820	.816	.812	.807	.802	.798	.793	.788
715	.878	.874	.871	.867	.863	.859	.855	.851	.847	.843	.839	.835	.830	.826	.822	.818	.813	.808	.804	.799	.794
720	.885	.881	.877	.873	.869	.865	.861	.857	.853	.849	.845	.841	.836	.832	.828	.824	.819	.814	.810	.805	.800
725	.891	.887	.883	.879	.876	.872	.867	.863	.859	.855	.851	.847	.842	.838	.834	.830	.825	.820	.816	.811	.806
730	.897	.894	.890	.886	.882	.878	.874	.869	.865	.861	.857	.853	.848	.844	.840	.836	.831	.826	.822	.817	.812
735	.904	.900	.896	.892	.888	.884	.880	.871	.871	.867	.863	.859	.854	.850	.846	.842	.837	.832	.827	.822	.817
740	.910	.908	.902	.898	.894	.890	.886	.881	.877	.873	.869	.865	.860	.856	.852	.848	.843	.838	.833	.828	.823
745	.916	.912	.908	.904	.900	.896	.892	.887	.883	.879	.875	.871	.866	.862	.857	.853	.848	.844	.839	.834	.829
710	.922	.918	.914	.910	.906	.902	.898	.893	.889	.885	.881	.877	.872	.868	.863	.859	.854	.849	.845	.840	.835
715	.928	.924	.920	.916	.912	.908	.904	.899	.895	.891	.887	.883	.878	.874	.869	.865	.860	.855	.851	.846	.841
760	.934	.930	.926	.922	.918	.914	.910	.901	.901	.897	.893	.889	.884	.880	.875	.871	.866	.861	.857	.852	.847
765	.941	.938	.932	.928	.924	.920	.916	.911	.907	.903	.899	.895	.890	.886	.881	.877	.872	.867	.862	.857	.852
770	.947	.943	.939	.935	.930	.926	.922	.917	.913	.909	.905	.901	.896	.892	.887	.883	.878	.873	.869	.863	.858
775	.954	.949	.943	.941	.938	.932	.928	.923	.919	.911	.911	.907	.902	.898	.893	.889	.884	.879	.875	.869	.864
780	.960	.958	.952	.948	.943	.939	.935	.930	.926	.921	.917	.913	.908	.904	.899	.895	.890	.881	.880	.875	.870

CHAPTER XI

SPUTUM

For the accurate examination of sputum it is necessary to give proper attention to its collection. The best container is a two-ounce, wide-mouthed bottle with a cork. Thoroughly sterilize both and keep sterile until used. Unless a twenty-four hour specimen is ordered, the sputum raised by the morning cough is preferred. If a fair representation of the usual morning sputum is not obtained, save from several coughs. Sometimes it is necessary to wait until the following morning for the specimen.

Have the specimen sent to the laboratory the same morning it is collected. If not possible to examine at once place in a refrigerator. For a routine examination it is unnecessary to measure the quantity unless it is a twenty-four hour specimen.

Be sure the specimen comes from the lungs or bronchi and not from the naso-pharynx. If collected following a deep expulsive cough its origin is quite certain. To prevent the sputum from mixing with cells from the upper air passages cleanse the nostrils and mouth with a physiological saline solution before collecting the specimen. If considerable mucus and squamous epithelial cells are found its origin is questionable as these come from the upper air passages.

Macroscopic Examination

Pour a portion of the specimen into a Petri dish or any convenient container spreading in a thin layer. Hold over black paper which greatly assists in the

examination. An ordinary paper plate painted black serves very well. Burn it as soon as the examination is completed.

Note the color and consistency of the specimen and the presence or absence of blood, mucus, food particles, Dittrich's plugs, concretions, and bronchial casts.

The **color** of the sputum is designated as colorless, yellow, yellowish-green, green, pink, red, reddish-brown, or black. Specify whether clear or opaque.

The **consistency** of sputum is classified as serous, mucous, purulent, sero-purulent, muco-purulent and bloody.

Blood is present either in streaks of fresh blood or mixed with the sputum which manifests itself as a pink or brownish-red color. The latter is often referred to as a "rusty" sputum. If in doubt concerning the presence of blood mix a small portion of the sputum with a little water and use the Benzidine test as outlined in testing for blood in the urine, with the exception that the sputum is not heated.

Dittrich's plugs are yellowish-gray particles of caseous matter varying in size from a millimeter to a centimeter in diameter. They are composed of a mixture of bacteria, fatty acid crystals, fat globules, and sometimes blood in which instance the color is reddish-brown. They usually have a putrid odor which is more pronounced when crushed. The plugs are formed in the bronchi and occasionally expectorated with the sputum but more often by themselves.

Concretions when found in the sputum are calcareous in nature and invariably from cases of tuberculosis.

Bronchial casts of various sizes are occasionally present. They are formed in the bronchi and when thrown off have a tree-like appearance. Unless containing blood they are grayish in color, being easily detected if the suspicious particles are floated in water in which instance they unfold.

Microscopic Examination

In making a microscopic examination of the sputum examine an unstained specimen as well as the stained one. The former is often omitted but is of great diagnostic value.

In preparing a slide for the unstained specimen select numerous suspicious looking portions, mount separately covering each with a cover-slip. Place under the low power and look for Curschmann's spirals, pigmented cells, elastic fibers and Charcot-Leyden crystals.

Curschmann's spirals are almost always present in the sputum obtained from a case of bronchial asthma. To the unaided eye they are just visible and appear as clear threads varying in length from 0.5 to 1.5 cm. When viewed under the low power a bright central fiber wound by many fine fibers is seen.

Charcot-Leyden crystals are often found in the sputum. They are slender and pointed at both ends, about 25 microns in length. They are associated with Curschmann's spirals and eosinophils. When the three are present it invariably means bronchial asthma.

Pigmented cells are large, flat cells of uncertain origin. Two kinds are observed; heart-failure cells stained with a blood-pigment called hemosiderin and carbon-laden cells.

Elastic fibers are always significant of some destructive lung condition. The greater the number of fibers the more serious the condition.

Search for necrotic portions and place a small amount on the slide. Press down with a cover-slip. Examine with the low power objective. The fibers are seen as wavy, slender threads sometimes occuring in bundles or a network.

The finding of fibrous tissue may be simplified by mixing a portion of the sputum with 10% sodium hydroxide. Let stand twenty-four hours and examine the sediment. If time is a factor centrifuge any time after waiting one hour.

When examining for **cells** stained smears are always preferred. Make a thin smear of sputum containing pus and use Wright's stain the same as for a differential blood count. Neutrophils in superabundance are present in the majority of cases but occasionally the percentage of lymphocytes or eosinophils is increased.

In the search for **bacteria** a stained specimen is imperative. Select a cheesy, purulent and necrotic particle if possible.

After finding the part to be used remove a portion with a platinum loop or sterile wood applicator. Place on the center of a slide or cover-slip and spread in all directions to make a thin smear. Air-dry and fix by passing over a flame or immersing in a 1 per cent solution of bichloride of mercury for three minutes. Wash in water, and stain. The Ziehl-Neelson method is the one usually recommended.

The Ziehl-Neelson Method

(a) Fix smear with heat for 45 seconds.
(b) Flood with carbol-fuchsin stain.
(c) Heat with low flame to steaming from 5 to 8 minutes, adding more stain as evaporation progresses. Do not over heat, and never allow smear to become dry.
(d) Wash with distilled water.
(e) Decolorize with acid-alcohol until the color has practically disappeared.
(f) Wash with distilled water.
(g) Counter stain with Loffler's alkaline methylene blue about 45 seconds.
(h) Wash with distilled water and dry over flame.

Examine the specimen under the oil-immersion lens to detect tubercle bacilli. Tuberculosis germs if present appear as red, thread-like rods occurring singly or in groups. On a properly stained slide they are easily recognized on the blue background.

Do not record the total number of germs found in the search but the average per microscopic field. If in all the fields a total of only one or two germs is found, make another slide or procure another specimen. If none are found, examine three or four other specimens collected on different days, to verify the result before a negative report is given.

In addition to tubercle bacilli, staphylococci, streptococci, pneumococci, influenza bacilli, and micrococcus catarrhalis are often present. A Gram stain greatly assists in their differentiation.

The **staphylococci** and **streptococci** are both pus forming organisms and many times found associated with tubercle bacilli constituting what is termed a

mixed infection. The staphylococci group together, while the streptococci grow end to end forming chains of various lengths. The **pneumococci** are the germs especially responsible for pneumonia. They occur in pairs enclosed in a gelatin capsule. They are ovoid and always with their ends together. **Influenza bacilli** formerly were thought to be the sole cause of influenza but this has never been proved. They are very small and within the pus cells. The **micrococcus catarrhalis** is a diplococcus of no great pathogenic importance. It takes a Gram-negative stain and may be intra- or extra-cellular.

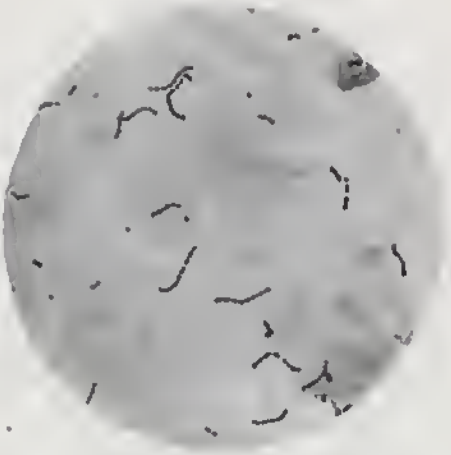

FIG. 42 — STREPTOCOCCI (Photo by Ziesel)

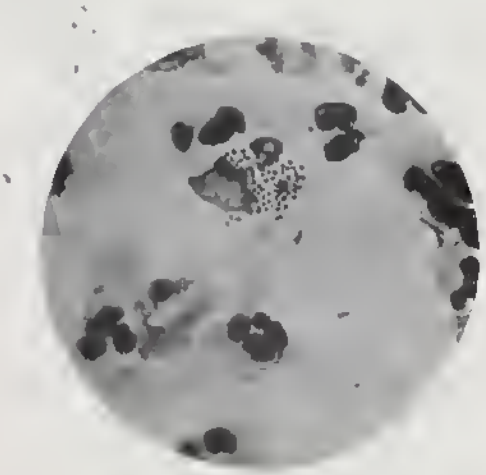

FIG. 43 — GONOCOCCI (Photo by Ziesel)

CHAPTER XII

PUS

The details of bacteriology will not be considered in this manual; nevertheless the subject should not be passed without receiving some attention. The average office technician ought to have a fair working knowledge of a few important bacteria, especially the following: Borrelia vincenti, staphylococci, streptococci, gonococci, tubercle bacilli, colon bacilli, and acidophilus bacilli.

The tubercle bacilli have been discussed in the chapter on Sputum while the colon bacilli and the acidophilus bacilli will be discussed in the chapter on Feces.

There are four kinds of germs which are most often found in pus: Borrelia vincenti, staphylococci, streptococci, and gonococci. The technique of their staining will be described but those desiring information dealing with their culture are advised to consult works on bacteriology.

Pus is a mixture of dead white cells, debris and bacteria. The cells are usually polymorphonuclear neutrophils although in some instances eosinophils are present. When desiring to know the kind of cells use the technique for a differential white cell count. This information is seldom desired except in cases of asthma. The main object in the examination of pus is to determine the various organisms present.

In staining any of the above germs, first make a smear by spreading some of the infected material upon the slide until a thin coating is made. Air-dry for a few minutes and fix by passing the slide over a

flame, film-side up. If too much heat is applied the smear will be spoiled for microscopical examination and if not enough, it will wash off. Experience will teach how much heat to use.

Some workers fix the smear by flooding it with methanol and leaving the alcohol on about five minutes. Then the slide is tipped so as to drain off the liquid and left to air-dry or it may be gently warmed until dry. Others use a 1% solution of bichloride of mercury to fix the smear. This is left on the slide for a minute or two and then washed off.

After the smear has been fixed, apply the stain directly to it. The length of time the stain remains is determined by its strength and kind. After staining, wash and dry the slide.

The **staphylococci** and **streptococci** take any good stain and show prominently on the slide. These two groups of bacteria are differentiated by the fact that staphylococci have a tendency to group while the streptococci join end to end like a chain.

The **gonococci**, the active agent in gonorrhea, are ovid cocci. They grow in pairs with their concave sides toward each other. They have a strong tendency to be intracellular. Occasionally some are found outside a pus cell but should receive no consideration unless some intracellular ones are found also. In acute gonorrhea they are easily found but in chronic cases it is more difficult as they are mixed with other pus organisms and constitute what is known as a "mixed infection".

The gonococci readily stain with Loeffler's methylene-blue but a positive diagnosis cannot be made with

such a stain. It is necessary to use a Gram stain and the germs must be Gram negative.

Some laboratories make two smears and stain the first with metylene-blue to find if intracellular diplococci are present. If found, the second smear is given a Gram stain to confirm the diagnosis. For a positive diagnosis, the cocci must be of the diplococcus variety, with their sides together, and must be intracellular as well as Gram negative.

The Gram Staining Method

(a) Fix smear with heat for 45 seconds, or cover with methanol for 3 to 5 minutes; in the latter case, pour off the alcohol and dry.
(b) Flood with crystal violet stain.
(c) Let remain 2 minutes.
(d) Wash with distilled water.
(e) Flood with Gram's iodine.
(f) Let remain 2 minutes.
(g) Wash with distilled water.
(h) Flood with acetone-alcohol (to decolorize) and wash with distilled water immediately.
(i) Treat one end of the smear with more of the decolorizer if necessary, and wash with distilled water.
(j) Counter stain with safranin for 45 seconds.
(k) Wash with distilled water and dry with heat.

The Borrelia vincenti spirochete, together with a characteristic fusiform bacillus, cause Vincent's angina. They produce abscesses and a false membrane in the mouth, along the gums and in the throat. The germs are so numerous it is necessary to wipe the ulcer with a little gauze before obtaining the material for the smear.

Borrelia vincenti are rather difficult to stain without a special staining solution. The following technique is very satisfactory.

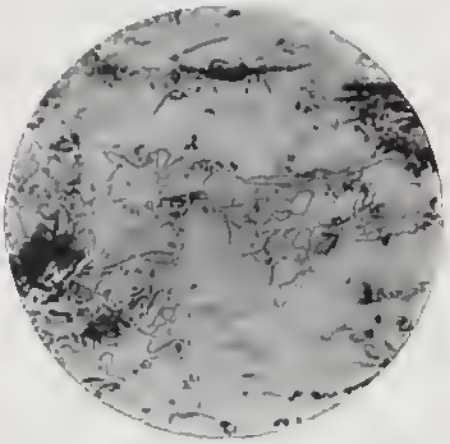

FIG. 44 — BORRELIA VINCENTI (Photo by Ziesel)

Borrelia Vincenti Staining

(a) Fix smear with heat as previously described.
(b) Cover smear with formalin gentian violet stain.
(c) Immediately add about the same amount of distilled water.
(d) Let stand about 3 minutes.
(e) Wash with distilled water and dry with heat.

CHAPTER XIII

FECES

A normal stool contains water, food-remnants, products of decomposition, bacteria, epithelium, mucus, enzymes and bile. If blood, parasites, ova, pathogenic bacteria or concretions are found the specimen is abnormal.

For accurate results the specimen should be free of urine and collected in a clean container. Examine as soon as possible after it is passed.

In most instances all that is desired from an examination of the feces is the determination of the presence or absence of parasites. However much valuable information is obtained by making a more complete examination.

The **color** of the stool is recorded as light, yellow, brown, black or clay-color.

The **consistency** may be expressed as liquid, soft, formed or hard. Observe carefully for any blood streaks.

The degree and kind of bacterial action determine the **odor,** which should be recorded as normal, offensive, putrid or sour.

Chemical Examination

The reaction of the stool in the majority of cases is slightly alkaline. It varies from alkaline to acid according to the diet and the preponderance of certain bacteria in the bowel. A meat diet stimulates the growth of putrefactive germs and renders the stool alkaline. Carbohydrates stimulate fermentive action and make the stool acid.

The reaction is expressed in terms of hydrogen-ion (pH) concentration. Use the following technique.

Feces Hydrogen-ion Concentration

(a) Place 10 gm. feces in a 100 c.c. beaker.
(b) Add 50 c.c. distilled water.
(c) Mix thoroughly by continued stirring until it reaches the consistency of thin paste.
(d) Filter and test the same as urine.

The Detection of Blood

(a) Mix a small portion of feces in water. Shake well.
(b) Use the Benzidine base test as for blood in urine.

This test is accurate and can be used when only a very small amount of occult blood is present. Blood from the descending colon or rectum is red and usually in streaks but very dark in color if from the stomach or small intestine. When in large quantities the stool is black and "tarry."

Mucus in large quantities usually signifies colitis. If present in small quantities and mixed with the stool it comes from the small bowel. Record the amount and distribution of the mucus.

Macroscopical Examination

In the search for **gall-stones** wash the fecal matter through a small-meshed sieve. The stones will remain in the sieve. To be sure the substance is a gall-stone, pulverize and dissolve in equal parts of ether and alcohol. Allow to evaporate. If a gall-stone, there will be cholesterol crystals which can be identified by the aid of a microscope. Another method is to add a drop of Lugol's solution to the sediment. If bile is present there will be a display of colors.

Many times it is advisable to search for worms Use the same sieve as for concretions. Round worms pin worms and segments of tapeworms, if presen ,

can easily be seen. To find pin worms collect the specimen following an enema; for round worms, after a vermicide and cathartic have been administered.

Microscopical Examination

When making a microscopical examination of the feces make smears from several portions of the stool for a reliable report. Special staining is necessary to diagnose parasites, ova, and bacteria. Pus cells, mucous shreds and epithelial cells can be seen without staining but their visibility is greatly improved by the stain.

The bacteria of the bowel can be divided into two general divisions, the Gram positive and Gram negative.

In infancy a Gram positive, acid producing germ called bacillus acidophilus predominates as long as the child is on a milk diet. Soon after weaning and milk becomes a minor portion of the diet the bacillus acidophilus germs rapidly diminish, being replaced by a Gram negative, alkaline producing germ of the colon bacillus type.

The proportional number of the above mentioned types of bacteria are quite accurately determined by making a Gram stain. They are roughly determined by ascertaining the pH of the stool. If acid, the Gram positive germs prevail and if alkaline the Gram negative.

The germs responsible for putrefaction belong to the Gram negative type. Replacing these with Gram positive bacillus acidophilus prevents the formation of toxic substances which so often are the causing factors of chronic disorders.

Another type of germs, the streptococcus, is present in the stool and often the direct cause of arthritis. It is Gram positive but readily differentiated from the bacillus acidophilus by its shape which is round while the latter is rod-like.

Gram Stain for Feces

(a) Thoroughly mix about 2 gm. feces with 50 c.c. water, and allow to settle.
(b) Make a smear from the sedm, wh'ch contains the germs.
(c) Fix by immersing in methyl alcohol for 5 minutes.
(d) Then follow the technique as given in the chapter on pus.

Parasites

The parasites inhabiting the bowel are classified as metazoa and protozoa. The former include the various worms already mentioned and the latter consists of unicellular animals. The protozoa exist in two forms, the active or vegetative, and the inactive or encysted.

In the active state they are in the semi-liquid portion of the bowel contents but change their form to the encysted type as soon as it becomes formed.

To examine the parasites in the active state give the patient a saline cathartic (never castor oil) and obtain a sample of the first liquid stool passed. Keep warm and examine on a warm stage as the protozoa are easily killed.

For the examination of the encysted type a portion of the above specimen is useless as the parasites never encyst outside the body. Obtain a specimen of formed stool. It is unnecessary to keep it warm as this type does not change its form when cooled.

Concentration of the cysts makes their detection easier. To do this, note the following technique.

Cyst Concentration

(a) Add to a few grams feces in a test tube 5 c.c. 5% acetic acid.
(b) Mix well, and strain through gauze.
(c) Mix the filtrate with an equal amount of ether.
(d) Centrifuge for three minutes.
(e) Four layers are seen. The cysts, if present, are in the bottom.

The diagnosis of the intestinal protozoa is made from both fresh and stained preparations. In the fresh smears the presence of the parasites is detected, and usually a tentative diagnosis can be made at this time. It is from the stained slides which reveal the nuclear and cytoplasmic characteristics that the definite diagnosis is made.

Heidenhain's iron-hematoxylin method of staining for the vegetative forms is highly recommended. For the cysts Kofoid uses an iodine-eosin stain which turns cysts a yellowish brown color while other objects take a pinkish color. To perfectly differentiate all the protozoa which inhabit the bowel much experience and a thorough training are necessary. In the average office laboratory no attempt should be made to make a diagnosis. The presence or absence of parasites can be determined and if present send the specimen to an experienced parasitologist.

The protozoa inhabiting the human bowel are of three types; ameba, flagellata and ciliata.

Of the amebas the three principle ones will be considered: Entameba histolytica, Entameba coli, and Endolimax nana.

The **Entameba histolytica** is the direct cause of amebic dysentery. The average diameter is 30 microns. It is very active having a typical amehoid movement. When seen in fresh warm smears it suddenly thrusts out hyaline, blade-like projections called psendopodia. It possesses many granules and only one nucleus which often is difficult to recognize. Usually several vacuoles are present which act as digestive organs. Blood cells may be seen in the vacuoles as they constitute the diet of the ameba. Therefore when making the smear look for blood-streaked mucus. One characteristic feature of Entameba histolytica is a clear granular-free band around the body which is more prominent in the end of the psendopodia.

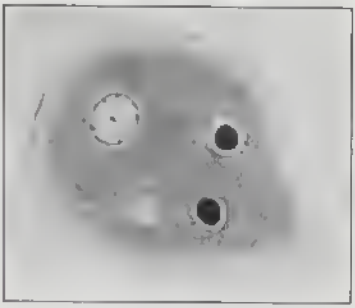

Fig. 45 — Entameba Histolytica (× 2000) Showing the Nucleus and Three Vacuoles Two of which Contain a Red Blood Cell

To stain without killing the ameba use a 1 to 10,000 solution of neutral red salt as suggested by Todd and Sanford. Use one drop of stain to a loopful of feces. The Entameba histolytica takes this "vital staining", but the Entameba coli does not.

They are active when in the semi-liquid portion of the bowel contents, but lose their activity becoming encysted when the contents begin to take definite form. As they change their form they become spherical in shape with a decided cell wall. The nucleus divides into four smaller ones. When the encystment is completed they are much smaller, being only one-half their original diameter.

The **Entameba coli** are more frequently found in the bowel than the Entameba histolytica. They are smaller and less active, but the nucleus is more prominent. They do not take the neutral red stain. Ingested red blood cells are seldom present but ingested bacteria are abundant. The Entameba coli are not so pathogenic as the histolytic variety, in fact most authorities claim they are non-pathogenic. Their cysts are somewhat larger than those of the Entameba histolytica. When mature they may have eight or more nuclei.

Endolimax nana is the smallest ameba found in the human bowel. The active state measures only 8 to 10 microns in diameter. The pseudopodia are hyaline, and the nucleus practically invisible in the living state. For these reasons it is often mistaken for a small Entameba histolytica. It is non-pathogenic. The cytoplasm is usually filled with food vacuoles containing bacteria and debris, but never blood. The cysts, generally oval in shape measure from 8 to 10 microns in length. The mature cysts contain four nuclei.

Of the **flagella** protozoans we have three main varieties, Giardia, Chilomastix and Trichomonas. Although easy to find they may be hard to differentiate.

They all have granules and are ovoid or pear-shaped with tail-like appendages called flagella, which serve as a means of locomotion. Ameboid movements are never present. Vacuoles are present which may or may not be contractile.

Giardia lamblia, the most common of the genus Giardia, is mildly pathogenic. It has a diameter of 12 to 20 microns and two nuclei. It is distinguished from the other two varieties by a large depression on one side with which it adheres to the bowel wall causing a mild colitis. Around the depression are three pair of flagella and one pair at its end. It lives in the small bowel and has the power to encyst itself upon its passage from the body.

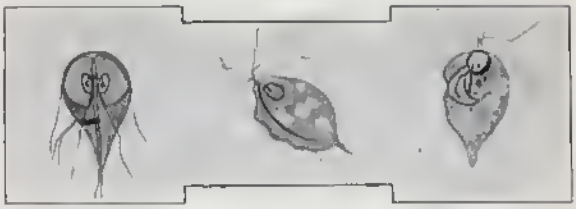

Fig. 46 — Left, Giardia Lamblia. Right, Chilomastix Mesnili. Middle, Trichomonas Hominis. (× 1000) (Drawn by Balch)

Chilomastix mesnili is the most often found of the genus in the human bowel. It is neither so common nor so large as Giardia lamblia. It has three flagella and a small opening to serve as a mouth. This parasite lives in the small bowel and is able to encyst itself when the stool becomes formed.

Trichomonas hominis is the most commonly seen of the genus Trichomonas. It is shorter than either the Giardia lamblia or Chilomastix mesnili and does not have the power to encyst itself. There are four flagella at the large end and a short one at the opposite end. One side has an undulating membrane. It is mildly pathogenic.

Under the third division of protozoa will be considered the **ciliata**. Of these only one is of sufficient pathogenic importance, the **Balantidium coli**, formerly known as Paramecium coli.

Balantidium coli are occasionally found in the large bowel of man and seldom overlooked because of their size, having an average length of 80 microns and a width of 60 microns. They are covered with short hair-like projections called cilia, the movement of which produces a very active locomotion. They possess two nuclei, a large and a small one. There are two contracting vacuoles and many granules. A small opening at one end serves as a mouth. They have the power to encyst themselves. In the active stage they penetrate the mucous membrane of the bowel, producing enough irritation to cause a diarrhea resembling dysentery. These and Entameba histolytica constitute the two main protozoa having a definite pathogenic action.

CHAPTER XIV

SUPPLEMENTARY TESTS

The preceding chapters have been devoted to the tests commonly used in the doctor's office. This supplementary chapter contains certain miscellaneous tests which the physician occasionally wishes the technician to make.

The Friedman Pregnancy Test (Modified)

The pregnancy test, which is about 98% accurate, is based on the detection of an anterior pituitary-like hormone in the urine of a pregnant woman. It has been detected as early as ten days after conception but usually is found after the sixth week. It is present in greater quantities in a morning specimen.

Procure a female rabbit at least fourteen weeks old and at least four pounds in weight. It must have been isolated for a period of three or four weeks just preceding the test, to insure against pregnancy.

Obtain a morning specimen of urine from the patient. Filter if cloudy. Take a small portion of it and test the acidity. If the urine is alkaline, slightly acidify with acetic acid. Warm the urine to 37 degrees Centigrade.

Cleanse one of the rabbit's ears and dilate the veins by rubbing with ether. Inject 10 c.c. of the warm urine into the marginal vein. If the vein does not stand out prominently, it will be easily located by holding the rabbit's ear over a microscope substage lamp from which the glass has been removed. Repeat the injection at the end of 24 hours.

Twenty-four hours after the second injection, perform a biopsy on the rabbit. If the test is posi-

tive there will be corpora hemorrhagica and corpora lutea in the rabbit's ovaries.

Sugar Tolerance and Sugar Retention

Instruct the patient to eat no food after seven P.M., and to come to the laboratory the following morning without breakfast, and with as little exertion or excitement as possible.

A specimen of urine is obtained for the testing of sugar, and a sample of blood taken from the patient's arm to test for the sugar content.

The patient now drinks 100 gm. of glucose (Karo), mixed with water and the juice of one or two lemons.

After one hour, urine and blood are again taken and tested for sugar. Repeat at the end of the second and third hours.

Urine shows no sugar normally. There may be a trace present at the end of the first or second hour following the glucose (dextrose) ingestion.

The readings for the blood sugar might normally be 100 mg. at the beginning, then rise to 135 or 150, and later return to 95 or 100.

If the blood sugar does not properly return to normal, or reaches the peak too quickly, or remains high too long, then the sugar tolerance is admittedly poor.

Some doctors may wish to modify the test as to the amount of sugar taken, and the intervals between obtaining the samples.

Blood Sedimentation Test, Westergren (Modified)

There are a number of good sedimentation tests. The one by Westergren is quite satisfactory and simple in technique.

The sedimentation tubes are 200 mm. internal length, and 2.5 mm. internal diameter. Ordinary glass tubing can be obtained, exactly the proper size.

Sterile sodium citrate, 3.8%, is used to dilute the blood according to the following technique:

Draw into a sterile 10 c.c. syringe a little over 1 c.c. of the sterile sodium citrate. Hold the syringe in a vertical position and push the plunger to the 1 c.c. mark, and attach the sterile needle.

Draw the blood, which mixes with the citrate, to the 5 c.c. mark. To thoroughly mix the blood and the non-coagulant, rotate the syringe several times.

Fill the sedimentation tube to the top, and let stand in a vertical position exactly one hour.

The normal sedimentation rate for men, measured from the bottom of the menisens of the clear liquid down to the top of the solid column of red cells, is 1 to 3 mm., and for women, 3 to 7 mm.

Anything greater than double these maximum limits should be considered pathologic. The greater the sedimentation rate or index, the greater the abnormality.

Blood Calcium. Tisdall's Method (Modified)

Have at hand 4% ammonium oxalate; 2% ammonium hydroxide; 1 N. sulphuric acid; N/10 potassium permanganate; exactly N/100 sodium oxalate; and redistilled water.

All glassware must be thoroughly cleaned with the following special solution: Dissolve C.P. sodium carbonate in concentrated sulphuric acid. The solution will get very hot. Clean all the glassware with this hot solution and rinse with redistilled water.

Take two graduated centrifuge tubes, and in one put 2 c.c. of the patient's clear blood serum and 2 c.c. redistilled water in the other. This second centrifuge tube is the "blank", used to determine the amount of calcium which enters the test from outside sources.

Add to each of the tubes 2 c.c. redistilled water and 1 c.c. of the 4% ammonium oxalate solution. Mix well, and let stand several hours, preferably over night.

Centrifuge each tube 10 minutes, and drain carefully. Wash the white precipitate (you may not see any in the blank tube) with 3 c.c. of the 2% ammonium hydroxide. This will not dissolve the calcium oxalate, but should remove all the excess, uncombined ammonium oxalate.

Centrifuge both tubes as before, and drain. Wash both tubes again, centrifuge and drain carefully.

Make up N/100 potassium permanganate by diluting the N/10 ten times, and titrate against the N/100 sodium oxalate to be sure the permanganate is exactly N/100, if not, make it exactly N/100.

Place 2 c.c. of the 1 N. sulphuric in each of the centrifuge tubes, mix well, dissolving the precipitate, and place in boiling water for one minute.

At the end of the minute turn out the fire, and begin titrating into the patient's centrifuge tube the

N/100 potassium permanganate from a 1 c.c. pipette, graduated to hundredths, until a beautiful lilac tinge appears and persists about a minute after thoroughly mixing.

Follow the same technique with the blank tube, noting carefully the hundredths of a cubic centimeter of the potassium permanganate required to neutralize it. Subtract this amount from the total amount required to neutralize the patient's tube.

One cubic centimeter of N/100 potassium permanganate is required to neutralize 0.2 mg. of calcium. Hence, the calculation would be as follows:

Suppose it took 1.08 c.c. to neutralize the unknown tube, and 0.06 c.c. to neutralize the blank. The actual amount of permanganate required to neutralize the 2 c.c. of patient's serum would then be 1.02 c.c. Then half this amount (the amount required to neutralize 1 c.c. serum) times 0.2 times 100 would equal 10.2, the milligrams calcium present in the patient's serum. The normal runs from 9 to 11 mg. in 100 c.c. serum.

Blood Inorganic Phosphorus, Benedict's (Modified)

Have at hand 20% trichloracetic acid; standard phosphate solution (3 c.c. equals 0.025 mg. P.); hydroquinone-bisulphite solution; and molybdic acid reagent.

Place 2 c.c. of the patient's serum in a test tube and add 4 c.c. of distilled water, and 4 c.c. of the 20% trichloracetic acid. Shake vigorously and let stand 10 minutes.

Filter through a dry quantitative analysis "ashless" filter paper into a dry test tube or small flask.

Place 3 c.c. of this filtrate into a sugar tube, and add 5 c.c. of distilled water. This is the unknown tube.

Into another sugar tube (the standard tube), place 3 c.c. of the standard phosphate solution, and 5 c.c. of distilled water.

Add to each sugar tube 1 c.c. of the hydroquinone-bisulphite solution, and 1 c.c. of the molybdic acid reagent.

Place both tubes, loosely stoppered in boiling water 10 minutes, and then in cold water 3 minutes.

Dilute each tube to the 25 c.c. mark with distilled water and read in the colorimeter.

Calculation: $\frac{\text{Standard}}{\text{Unknown}}$ x 4.166 equals mg. inorganic phosphorus in 100 c.c. blood serum. The normal range is 3.2 to 4.3.

Van den Bergh Test (Tannhauser and Anderson Modification)

Take 2 c.c. of clear blood serum and add 1 c.c. freshly prepared sulphanilic reagent (mixture of sulphanilic acid and sodium nitrite).

There may be a decided change of color. If so, it will not be necessary to split off the bilirubin by use of alcohol, but the unknown solution may be compared at once in the colorimeter with the standard solution (represents 0.5 mg. bilirubin in 100 c.c.).

Otherwise, add 2 c.c. of saturated ammonium sulphate and 10 c.c. of alcohol (95%).

Centrifuge and compare with the standard in the colorimeter.

Calculation: $\frac{\text{Standard at (20)}}{\text{Unknown}}$ x 3.75 equals mg. bilirubin in 100 c.c. of blood serum.

The Kahn Flocculation Test For Syphilis

The test depends upon the appearance of a precipitate (provided patient is positive) when the antigen comes in contact with the patient's serum.

Kahn antigen must be used. Dilute at the time of running the test by mixing with physiologic saline according to the proportions (titre) on the antigen bottle. This makes antigen emulsion.

Use Kahn test tubes, and mix by pouring the saline into the antigen first, then back and forth 20 times. Let stand 20 minutes.

Arrange a row of three test tubes (Kahn), from left to right, across the front of the test tube rack and call it row "A".

Behind this "A" row, arrange a second similar row, and call it row "B". Place a third row back of row "B" and call it row "C".

The left hand, or first tube, in the A, B, and C rows is for the positive serum. The second tube in each of the three rows is for the negative serum. These two sera are for controls to check the accuracy of the technique and the quality of the chemicals. The right hand tube in each of the three rows is for the patient's serum.

The positive serum, the negative serum, and the patient's serum must be "inactivated" before running the test by placing in a water bath at 57 degrees C. for 30 minutes.

Using a Kahn pipette (graduated to thousandths), carefully pipette 0.050 c.c. of the antigen emulsion into the three A tubes. Pipette completely down to the bottom of the tube.

Now pipette 0.025 c.c. emulsion into each of the B tubes.

Pipette 0.0125 emulsion into the C tubes.

Using a 1 c.c. pipette, place 0.15 c.c. negative serum into the front, middle, and back tubes devoted to the negative control.

Blow out the pipette, placing the tip against a towel to absorb all the fluid, and using the same pipette, place 0.15 c.c. of the patient's serum in each of the three tubes devoted to the unknown.

Again blow out the pipette, and place 0.15 c.c. of the positive serum in each of the three positive control tubes.

If this order is not followed, it will be absolutely necessary to carefully clean the pipette each time, or use another to avoid contamination.

Shake all the tubes (all at the same time) four minutes.

Place in a water bath at 37 degrees C. for 15 minutes.

Add 0.5 c.c. saline to all of the nine tubes. Sometimes another cubic centimeter of saline is also added

to each tube to have more fluid in the tubes to aid in the examination under the low power of the microscope.

Hold to the light, or before a magnifying glass, or under the low power of the microscope, and the positive tubes should show a good flocculation.

A four plus (++++) will show a good precipitate, perhaps some clumps, in a clear fluid.

A three plus (+++) reaction will be similar to the first, but the fluid will not be quite clear.

A two plus (++) reaction will show a precipitate in a cloudy fluid.

A one plus (+) reaction will show a smaller amount of precipitate in the cloudy fluid.

A negative reaction will show no precipitate.

The Ide Syphilis Test

This is a rapid test, operating on the principle of agglutination. It is quite reliable for testing blood or serum which proves to be negative. If the Ide test shows a strong agglutination, check with a Wassermann or other additional tests.

For the test, procure an Ide Test Kit and carefully follow the printed directions which accompany it.

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